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ELECTRIC STIMULATION OF PLANT GROWTH[1]

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INTRODUCTION

The use of electricity as a means of increasing plant growth is a subject of perennial interest. A year seldom passes without the announcement of a new method by which electric forces are to be utilized to increase the yields of agricultural plants.

Electricity has proved to be such a convenient and serviceable form of energy in many human activities that it seems not unreasonable to hope for a method of utilizing it in plant production. This hope is strengthened by the fact that light and electricity are closely related phenomena and that light is one of the important factors in plant growth.

Consideration of the atmospheric potential gradient to which all plants are exposed gives an added reason for associating electrical currents with growth. The air has been found to be electrically charged with respect to the earth, and the difference in potential increases with height. It follows from this that plants in nature, growing in the earth and with their tops in the air at some distance above the earth, are constantly being traversed by minute but measurable currents of electricity. What could be more reasonable than to expect that an increase or decrease of this current should affect the growth of the plant?

In most of the new methods proposed the energy is drawn from the air by utilizing the atmospheric gradient. None of these methods have succeeded in other hands than those of the originator, and inventions claiming to increase plant growth by utilizing the atmospheric gradient may properly be classed with water witches, sex determiners, and perpetual-motion machines.

There is, however, from careful and capable British investigators, a body of evidence interpreted by them as demonstrating that increased plant growth follows electric excitation of a certain intensity and duration supplied from a charged network suspended above the plants. These investigations, begun by Sir Oliver Lodge as field experiments, have been continued by V. H. Blackman as laboratory experiments under carefully controlled conditions.

Shortly after the results of Lodge's field experiments were announced the Bureau of Plant Industry undertook the verification of these results. An experiment that duplicated in all essential particulars the "set-up" used by Lodge was undertaken. For 10 seasons treated and untreated plots were compared, but there was no measurable

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difference associated with the treatment.[2] It was realized that some detail in equipment might have been overlooked or that the treatment brought about a change in some condition of growth that was a limiting factor in the English experiments but was not a limiting factor under the very different environment of Washington. On the contrary, it was possible that the differences obtained in England on one series of plots of untested uniformity might have been due to causes other than the treatment.

When Blackman subsequently reported positive results from laboratory comparisons it seemed desirable to determine whether a duplication of these more carefully controlled English experiments would give similar results under the conditions prevailing at Washington. In Blackman's experiments electric stimulation was shown to be rather capricious. It would appear that, to get anything approximating uniform behavior, the strength of the field must be controlled within narrow limits and the treatment must be restricted to a certain part of the day. The greatest stimulation appears to take place after the treatment has been discontinued. These restrictions suggest that the observed differences, which are of undoubted statistical significance, may have been due to uncontrolled factors of the environment that obtained in this particular set of trials.

The method used by Blackman was to prepare a series of pots of uniform size, fill them with the same soil, plant them with a uniform lot of seed, and then separate them into groups of 4 to 10 each. One of these groups was maintained as a control, and the others were subjected to potential gradients of known intensity by means of an electrically charged overhead network.

There are two considerations that make it especially difficult accurately to compare plants treated electrically with their untreated controls. The first is that the discharge from an overhead network can not be confined easily to the area immediately below the network. This is especially true if air currents are passing over the treated area. The second obstacle is that there is assumed to be a residual effect of the treatment that persists for an indefinite period after the treatment is discontinued. If this assumption is given weight it precludes the possibility of using the same area alternately as treated and control.

METHODS

In the laboratory two methods of detecting the effects of a given treatment are available: (1) Similar plantings can be divided into treated and control and both exposed to the same environment as nearly as possible, except for the factor under investigation; or (2) the growth of an individual or group of individuals under normal conditions can be observed for a given period. The treatment is then

[2] The results of field experiments conducted by the Bureau of Plant Industry are reported in the following publication: BRIGGS, L. J., CAMPBELL, A. B., HEALD, R. H., and FLINT, L. H., ELECTROCULTURE. U. S. Dept. Agr. Bul. 1379, 35 p., illus. 1926. This publication may also be consulted for a bibliography of electrocultural experiments. The most important contribution to this subject that has appeared since Bulletin 1379 was issued is the following publication: LIPPERHEIDE, C., NEUERE UNTERSUCHEN ÜBER DEN EINFLUSS DER ELEKTRIZITÄT AUF PFLANZEN. Angew. Bot. 9: 561–625, illus., 1927. The method described in this article involved the use of chambers in which the air was subjected to conditions of different ion content. The results were interpreted as indicating definite and appreciable growth increases associated with increase of ions in the air. These investigations are not comparable with the trials herein reported. The method of enhanced atmospheric electrical potential gradient used in the writers' experiments has the effect of increasing the ions available to the plant in unit time, but places restrictions upon the movement of these ions.

applied, and if a change in rate follows it is ascribed to the treatment. There are some rather serious objections to the latter method. Growth rates, especially during seedling stages, are not uniform even under uniform conditions, but show progressive changes. It is necessary, therefore, to determine in advance the form of the normal growth curve and to compare the growth during treatment, not with the growth for the preceding period, but with the growth expected if the normal growth curve had been followed throughout the treated period. Individuals differ in the form of their growth curves, as in other particulars and there is no way of knowing how closely the particular individual treated conforms to the normal or average growth curve. Furthermore, the method is laborious and the results are not readily amenable to biometrical tests of significance. For these reasons the method of comparing similar cultures was adopted in the writers' experiments. With this method duplication is easy and the biometrical treatment of results is simple and direct.

The electroculture experiments here reported were carried out in the Biophysical Laboratory of the Bureau of Plant Industry at Washington, D. C. Each experiment consisted in subjecting a group of seedlings during their early period of development to conditions of modified atmospheric electrical gradient and at the same time in holding a similar group of seedlings to identical environmental conditions except for the presence of the normal atmospheric electrical gradient.

In the initial trials the seedlings were grown in groups of 100 and spaced 1 inch apart in each direction in wooden boxes of coarse sand. When the young plants were about 1 cm. high the two most uniform and similar boxes of the four planted were selected for the experiment. These were placed upon parallel metal bars in two grounded metal cages used for the trials. The two cages, which were identical in structure, were placed upon the same table in the laboratory. The supporting bars for the boxes and the supports of the adjustable metal screens suspended above the plants were mounted in sulphur insulating blocks. In one cage this overhead screen was attached to a source of high-voltage direct current, and the strength of the current passing through the plants was modified by the raising or the lowering of the screen. The current was measured with a sensitive galvanometer, calibrated previous to measurement, and placed in a line connecting the moist soil in the box with the ground. A storage battery, an induction coil, and a Lodge-valve "hook up" permitted the passage of the desired range of current strengths with a space of several centimeters between the plants and the screen. The treatment was not confined to the same cage throughout the experiments, but was changed several times during the course of the experiments. The light conditions within the two cages were equalized as far as possible by the adjustment of overhead electric lights to identical resistance values of a photo-electric cell. The light conditions were subnormal and caused the plants to elongate somewhat, but the foliage was normal in color. The set-up for these experiments is illustrated in Figure 1, which shows the two cages with one side removed to expose the interior arrangements.

Various methods of watering were tested, the object being to maintain uniform moisture conditions in the soil of the several boxes.

The method finally adopted was, after planting, to place all the boxes in water until the soil was thoroughly soaked. As soon as the surplus water had drained away the boxes were placed in their final positions. With the type of soil used enough water usually was held in the soil to supply the plants until the end of the experiment without further watering. Weighing the boxes before and after immersion showed the range of moisture content to be less than 3 per cent.

EXPERIMENTS IN 1924 AND 1925

The experiments were begun in December, 1924. Between this date and July, 1925, 36 experiments were completed, 24 with maize and 12 with barley. A current of 10^{-9} amperes per plant, which had been found by Blackman to give the most promising results, was employed with few exceptions. In some of the experiments the treatment was continuous, and in others the current was applied only during either the day or the night. The results are given in Tables 1 and 2. In most of the experiments the difference between the treated and the control was clearly significant, but the control exceeded the treated about as often as the treated exceeded the control. Evidently unknown variables were as potent as the treatment.

TABLE 1.—*Results obtained in the study of the influence of an electric current on the growth of maize seedlings in 1924 and 1925*

Experiment No.	Treatment (amperes per plant)	Time of application	Treated plants		Control plants		Difference (treated−control)	Difference÷probable error	Treated÷control
			Mean height (cm.)	Standard deviation (cm.)	Mean height (cm.)	Standard deviation (cm.)			
1	-10^{-9}	Day	4.09 ±0.090	1.296	4.35 ±0.105	1.486	−0.26 ±0.138	1.88	0.940
2	-10^{-9}	do	5.03 ± .159	2.328	6.81 ± .145	2.126	−1.78 ± .215	8.28	.739
3	$+10^{-9}$	do	7.18 ± .155	1.549	7.21 ± .149	1.565	− .03 ± .215	.14	.996
4	$+10^{-8}$	do	23.80 ± .255	3.772	24.37 ± .275	4.014	− .57 ± .375	1.52	.977
5	$+10^{-7}$	do	11.164± .120	1.750	11.391± .135	1.976	− .227± .181	1.25	.980
6	$+10^{-7}$	do	7.319± .068	1.000	7.306± .071	1.044	.013± .098	.13	1.002
7	$+10^{-6}$	do	8.858± .184	2.726	9.846± .189	2.774	− .988± .264	3.74	.900
8	$+10^{-10}$	do	11.72 ± .180	2.819	11.700± .172	2.511	.02 ± .249	.08	1.002
9	$+10^{-9}$	Night	12.707± .125	1.843	11.920± .121	1.798	.787+ .174	4.52	1.066
10	$+10^{-9}$	do	8.816± .094	1.379	7.927± .160	2.336	.889± .186	4.77	1.112
11	$+10^{-9}$	do	8.404± .174	2.573	7.616± .174	2.578	.788± .246	3.20	1.103
12	$+10^{-9}$	do	12.903± .123	1.772	12.583± .139	2.024	.320± .186	1.72	1.025
13	$+10^{-9}$	do	8.979± .152	2.226	8.632± .090	1.312	.347± .357	.97	1.040
14	$+10^{-9}$	do	8.740± .079	1.172	7.320± .149	2.218	1.42 ± .169	8.40	1.194
15	$+10^{-9}$	do	10.380± .223	3.037	10.822± .224	3.249	− .442± .316	1.40	.959
16	$+10^{-9}$	do	10.285± .212	3.010	10.451± .231	3.313	− .166± .314	.53	.984
17	$+10^{-9}$	do	11.342± .106	1.514	10.770± .122	1.725	.572± .162	3.53	1.053
18	$+10^{-9}$	do	8.388± .255	2.459	7.193± .236	2.331	1.195± .347	3.44	1.166
19	$+10^{-9}$	Day	17.690± .227	3.212	15.217+ .269	3.817	2.473± .352	7.02	1.162
20	$+10^{-9}$	do	13.077± .370	3.617	14.165± .560	5.369	−1.088± .671	1.62	.923
21	$+10^{-9}$	Day and night	8.324± .203	1.956	9.542± .225	2.114	−1.218± .303	4.02	.872
22	$+10^{-9}$	Day	11.004± .425	4.071	13.644± .348	3.348	−2.640± .549	4.81	.806
23	$+10^{-9}$	do	21.449± .357	3.347	18.292± .484	4.687	3.157± .601	5.25	1.172
24	$+10^{-9}$	Day and night	16.735± .215	2.980	18.222± .245	3.280	−1.487± .326	4.56	.918
Mean			11.182	2.392	11.137	2.625			1.004

TABLE 2.—*Results obtained in the study of the influence of an electric current on the growth of barley seedlings in 1924 and 1925*

Experiment No.	Treatment (amperes per plant)	Time of application	Treated plants		Control plants		Difference (treated−control)	Difference ÷ probable error	Treated ÷ control
			Mean height (cm.)	Standard deviation (cm.)	Mean height (cm.)	Standard deviation (cm.)			
1	$+10^{-9}$	Day	9.948±0.138	2.023	8.328±0.252	2.496	1.620±0.287	5.6	1.194
2	$+10^{-9}$	do	7.669± .308	2.858	7.184± .308	2.831	.485± .436	1.1	1.068
3	$+10^{-9}$	do	8.000± .160	1.529	7.988± .092	1.293	.012± .184	.1	1.002
4	$+10^{-9}$	do	9.405± .148	1.510	9.165± .145	1.519	.240± .210	1.1	1.026
5	$+10^{-9}$	do	10.572± .156	1.554	9.560± .160	1.608	1.012± .223	4.5	1.106
6	$+10^{-9}-10^{-5}$	Night	9.628± .196	1.645	10.907± .170	1.762	−1.279± .259	4.9	.883
7	-10^{-9}	do	9.914± .331	3.318	12.792± .344	3.101	−2.878± .478	6.0	.775
8	$+10^{-9}$	Day	8.803± .172	1.741	10.219± .135	1.959	−1.416± .190	7.4	.861
9	$+10^{-9}$	do	12.399± .274	2.767	9.573± .327	3.072	2.826± .427	6.6	1.295
10	$+10^{-9}$	Night	7.876± .126	1.835	6.074± .120	1.741	1.802± .174	10.4	1.297
11	$+10^{-9}$	Day	11.246± .315	2.562	10.442± .173	2.498	.804± .352	2.3	1.077
12	$+10^{-9}$	do	8.607± .308	2.894	10.868± .276	2.721	−2.261± .414	5.5	.792
Mean			9.505	2.186	9.425	2.217			1.031

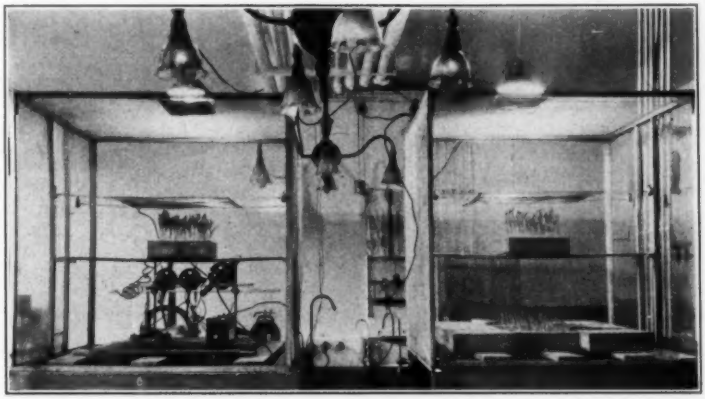

FIGURE 1.—Apparatus used in electric treatment of growing plants in 1924 and 1925. One side of each cage has been removed to expose the interior arrangements

An examination of the various combinations of time of treatment and intensity of current showed the most favorable combinations to be in the group of experiments in which maize was treated with a current of 10^{-9} amperes per plant during the night hours. There were 10 experiments of this kind, and in 8 the mean height of the treated was greater than that of the control. In three of these the difference was over four times the probable error. In the two experiments in which the control was larger than the treated the difference was insignificant.

Disregarding the probable errors of the individual experiments, the array of the 10 differences examined by Student's [3] method showed that, due to chance, a result as favorable as this was not to be expected more often than once in one hundred times.

[3] ANONYMOUS. THE PROBABLE ERROR OF A MEAN. By Student. Biometrika 6: 1–25, illus. 1908.

The significance of the mean difference was somewhat lessened by the fact that this group of experiments, showing the greatest mean difference, was chosen from among a series of such groups. Had the series been sufficiently extensive an individual departure of the observed magnitude would have occurred as a chance departure. At any rate the results were encouraging, and it was planned to conduct a more carefully controlled series, repeating this combination of maize treated at night with a current of 10^{-9} amperes per plant.

EXPERIMENTS IN 1926

With stationary cultures it seemed possible that the results might be influenced by environmental differences associated with the locations of the boxes. To eliminate this possible source of error, all cultures, both control and treated, were placed on a slowly revolving clinostat. Suitable connections were provided to permit an electrical treatment corresponding to that of the early trials. This arrangement is shown in Figure 2.

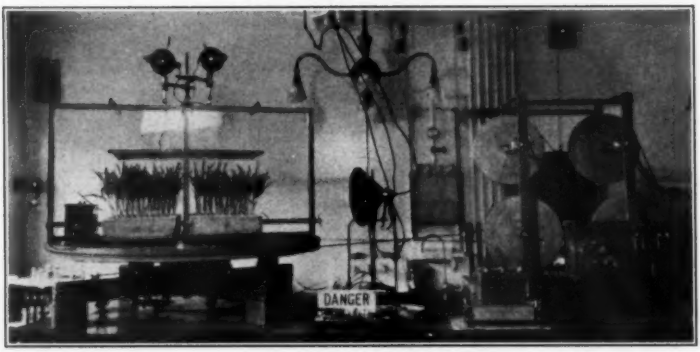

FIGURE 2.—Apparatus used in electric treatment of growing plants in 1926, 1927, and 1928

In this series of trials the current was taken from an alternating-current generator and stepped up with a large induction coil. From this coil it was passed through rectifying Lodge valves to the adjustable screen suspended above the plants. Additional light was furnished through the installation of 100-watt daylight lamps directly over the clinostat.

Sandy loam was substituted for the sand of the earlier trials. The number of boxes was increased from two to four. This gave two treated and two control boxes and made it possible to estimate the chance variations in box means.

The growth of the plants during a night's treatment shortened the distance to the screen, and the plants were thereby subjected to increased current. By taking readings of the amperage at the beginning and at the end of a treatment period, however, it was possible to estimate the range of this effect and to compensate for it in a measure by reducing slightly the current flow at the beginning of the subsequent treatment. By this means the fluctuations in current were controlled within narrow limits. Each of the experiments

extended over a period of about two weeks from the planting of the seeds. The plants were treated 6 to 10 days.

In the 1926 experiments the mean elongation instead of the mean final height of the seedlings was used as an index of plant response to treatment. The mean elongation was computed by subtracting the mean initial height from the mean final height. A comparison of the variability of the seedling elongation with that of final height, in boxes having the same treatment, indicated that the large differences in final height of similarly treated boxes, were associated with differences in the initial height. This was doubtless the case in the early experiments when initial height was not measured.

Since the boxes of any experiment were all prepared and treated alike, significant differences in initial height were not to be expected, and the reason for their occurrence is an interesting problem, which is being investigated. Whatever the causes of such differences, it is obviously desirable to eliminate them from comparisons of the effect of subsequent treatment.

To restrict the comparison to the elongation made during treatment does not completely eliminate the effect of diversity in initial height, since there is usually a slight correlation between initial height and elongation. When based on individual plants, this correlation, though small, is always positive in sign, but when based on box means it is usually insignificant and may be of either sign.

In 1926, 10 experiments were tried with maize seedlings treated with a current of 10^{-9} amperes during the night. The mean differences in elongation between treated and control are given under the column heading "Treated − control" in Table 3. In 7 of the 10 experiments the treated made a greater elongation than the control, and in at least one of these the difference is too large to be ascribed to chance. On the other hand, in one of the three experiments in which the control exceeded the treated the difference is also significant.

TABLE 3.—*Differences in growth rates associated with the electric treatment of maize seedlings with a current of 10^{-9} amperes applied at night compared with differences associated with an irrelevant factor*

Experiment No.	Treated − control			High number − low number *		
	Mean difference in elongation (mm.)	Probable error	Difference ÷ probable error	Mean difference in elongation (mm.)	Probable error	Difference ÷ probable error
1	6.0	4.5	1.3	−2.0	4.5	0.4
4	1.3	3.4	.4	−3.0	3.3	.9
5	10.2	3.4	3.0	−17.0	2.8	6.1
6	23.9	2.6	9.2	−8.2	2.8	2.9
8	3.1	1.9	1.6	−4.1	1.9	2.2
9	6.8	3.3	2.1	.2	3.2	.1
11	−1.5	1.9	.8	−2.4	1.7	1.4
13	−14.7	2.2	6.7	−7.9	2.2	3.6
20	−3.3	2.5	1.3	1.0	2.5	.4
21	11.1	3.3	3.4	−17.2	3.2	5.4
Mean	4.29	2.18	2.0	−6.06	1.39	4.36

* See p. 592 for explanation of high number and low number.

The mean of the array of differences, treated − control, is 4.29 ± 2.18, $t = 1.33$. The probability of this being due to chance is about

one in five.[4] Notwithstanding the great care exercised to insure uniformity of exposure, there were many instances in which boxes similarly treated showed significant differences in the mean elongation. This suggested that the observed individual differences between treated and control, although of undoubted significance statistically, might have but a chance association with the treatment.

To determine whether similar differences might occur when no electrical treatment was involved, the boxes of each of the 10 experiments were divided in such a way as to place one treated and one control box in each pair.

It will be recalled that in each experiment four boxes were used, of which two were treated and two were controls. In making the final comparison the individual measurements of the two treated boxes were combined into a single population, which was compared with the combined population of the two control boxes. It was now proposed to return to the original measurements and again combine them into two populations, each of which would consist of a treated box combined with a control.

In making the combinations it was necessary to devise a method for selecting at random the treated and control boxes to be combined. It was thought that a completely random choice could be effected by utilizing the serial numbers that had been stenciled on the boxes when they were made.

In starting the original experiments the boxes had been taken without regard to the stenciled number, and it was not until the boxes were segregated into treated and control that the box numbers were entered in the record. The plan now adopted was to combine the measurements of the treated box having the highest stenciled number with those of the control box having the highest number. As an example, experiment 1 was grown in boxes 1, 7, 10, and 11, of which 1 and 7 were treated and 10 and 11 were controls. Box 7 was now combined with box 11, the combined population was designated "high number," and boxes 1 and 10 combined were designated "low number." A similar procedure was followed in each of the 10 experiments, and the mean of the high number minus the mean of the low number with the probable error of the difference was calculated for each. These mean differences gave a series similar to that in which the treated was compared with the control. The results are given in Table 3 under the heading "High number − low number."

The individual differences do not show such a wide range as in the original series, although in two experiments the difference is more than five times the probable error, but the mean of the array of differences is -6.06 ± 1.4. The statistical significance of the mean difference calculated by Student's method gives $t = 2.9$ and $P = 0.01$, or odds of 1 in 100.

Taken at their indicated value, it would be necessary to conclude that the numbers that happened to be stenciled on the boxes in which the seedlings were grown had influenced the rate of growth more than did the electric treatment.

The clearly significant difference in favor of boxes bearing low numbers is an illustration of the difficulty of eliminating uncontrolled

[4] For the method of deriving the probability, see: FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS, p. 106–110. Edinburgh and London. 1925.

factors that may be associated with the factor under investigation. Had a difference of such unquestionable significance been obtained when the boxes were divided into treated and control, it might have been thought superfluous to question the conclusion that the effect of treatment had been demonstrated. With the division made on an utterly irrelevant difference, such as the ordinal standing of the labels, it is known, of course, that some uncontrolled factor must have been associated with the arbitrary classes. In this particular instance the difference probably is to be explained by the fact, subsequently established, that certain boxes exercise a deleterious effect on the growth of seedlings. These boxes chanced to bear high numbers and were used in a sufficient number of experiments to bias the results. At the time the 1926 experiments were made, differences in the boxes themselves had not been thought of as even a possible factor. The boxes were of the same dimensions, were made from the same stock of pine lumber, and were to all appearances identical. It was shown subsequently that the boxes having a deleterious effect on growth lost water more rapidly than other boxes.

In view of the significant mean difference in elongation associated with particular containers, it is obvious that no confidence should be placed in the much smaller difference associated with the electric treatment. On the other hand, it would not be permissible to conclude from these results that the treatment was without effect. The mean elongation of the plants in the control boxes in the 10 experiments was 65.44 mm., and since the probable error of the mean difference was 2.18, an increase or decrease in the mean elongation of less than 15 per cent would not have inspired confidence in the reliability of the difference.

Of the 1926 results the most that can be said is that the treatment did not influence the elongation of seedlings by as much as 15 per cent.

It would be necessary to make approximately 20 repetitions of this type of experiment to detect a difference of even 10 per cent with reasonable certainty. To avoid this unsatisfactory expedient, a further effort was made to obtain a more uniform behavior of similarly treated boxes. The analysis just reported indicated that a part of the diversity was associated with the box itself. That certain boxes ran either low or high with a measurable consistency was verified by direct experiment.

There were selected six boxes that showed no correlation with seedling growth. Efforts to obtain uniform growth with the use of these boxes were continued by taking still greater care in the mixing and distribution of the soil. The planting was further standardized by using for planting a mechanical device that placed all seeds at a uniform depth and in the same position.

Some of these refinements seem to have removed the disturbing factors, and two experiments were completed in which all six untreated boxes behaved with a satisfactory degree of uniformity. A comparison of the variance of row means with that of box means, using the method proposed by Fisher,[5] showed the variance in the means of the boxes to be no greater than was to be expected from the variance of the means of the rows.

[5] Fisher, R. A Op. cit., p. 192-194.

EXPERIMENTS IN 1927 AND 1928

The procedure adopted for the experiment of 1927 and 1928 was to start six boxes on a large clinostat and, when the plants were ready for the initial measurement, to select the four boxes with the most uniform initial height, to pair them in a way to make the mean height of the pairs as nearly identical as possible, and to apply the treatment to one of the pairs chosen at random.

The first step in analyzing the results of an experiment was to compute the means and standard deviations of the elongation of the plants of each box separately. The uniformity of the two treated boxes and that of the two controls was then examined by comparing the difference in the means with the standard error of the difference. If neither the treated boxes nor the controls showed a difference between the two boxes exceeding twice the standard error, the individual measurements of elongation of the two treated boxes were combined into a single population and the mean and standard deviation calculated. The measurements of the two control boxes were similarly treated.

With this improved technic five experiments were run with no significant differences between the means of duplicate boxes and with standard deviations sufficiently low to detect mean differences of the order of 5 per cent. The results are shown in Table 4.

TABLE 4.—*Effect of electric current on maize seedlings as measured by various indices of growth*

Experiment No.	Initial height (mm.)		Character measured	Final measurements			
	Treated seedlings	Control seedlings		Treated seedlings	Control seedlings	Treated − control	Difference ÷ probable error
125	76. 17± .41	75. 00± .43	Final height (mm.)	157. 9 ±1. 76	155. 4 ±1. 88	2. 5 ± 2. 6	1. 0
125	76. 17± .41	75. 00± .43	Dry weight (mgm.)	690. 2 ±8. 95	652. 4 ±9. 55	37. 8 ±13. 1	2. 9
126 [a]	57. 96± .42	59. 83± .42	Green weight (mgm. per plant).	801. 2 ± .90	852. 5 ± .92	−51. 3 ±12. 8	4. 0
127	49. 38± .50	49. 62± .44	do	806. 3 ±7. 2	814. 6 ±6. 7	− 8. 3 ± 9. 8	.8
128	39. 48± .27	39. 89± .29	Elongation (cm.)	15. 54± .07	15. 52± .07	.02± .099	.2
129	50. 94± .32	51. 45± .35	do	18. 24± .08	18. 25± .09	−. 01± .12	.1
130 [b]	22. 86± .22	22. 82± .21	do	13. 19± .07	13. 40± .07	−. 21± .10	2. 1

[a] The control of the current was defective and for a part of the time the treated plants received more than the prescribed amperage.

[b] The treated plants were given the maximum current possible without sparking.

All of the plants used in experiments 125, 126, and 127 were treated with 10^{-9} amperes at night, and the comparison was based on weight instead of elongation. In experiment 125 the plants from each row were oven dried and the box means and standard deviations determined from the mean dry weight per row. In later experiments green weights were used. This was made possible by a simple device by which an entire row of plants could be cut with one movement. As each row was cut it was placed in a closed weighing can, and as soon as the harvesting of a box was completed the cans were weighed and the net green weight was recorded. With this technic green weight proved a more sensitive and reliable measure than dry

weight, though not so sensitive as comparisons based on individual plant elongation.

The experiments based on weight serve to show that the electric treatment did not increase the weight of the aboveground parts of the plant by as much as 8 per cent. Experiment 126, which showed an apparently significant decrease in green weight, may be disregarded, since the overhead network slipped and for a part of one night gave a greatly increased current with enough sparking to injure some of the plants.

Experiment 127 provided the most satisfactory test based on weight. Here the control exceeded the treated plants by an insignificant amount, and a difference of 5 per cent could have been detected with assurance.

Experiments 128 and 129, in which the treated boxes were also given 10^{-9} amperes at night, afford conclusive evidence that under the conditions of these experiments the treatment produced no measurable effect on elongation. The plants were remarkably uniform, and the treatment ran smoothly. If the rate of elongation had been either increased or decreased by as much as 3 per cent the difference would have been apparent.

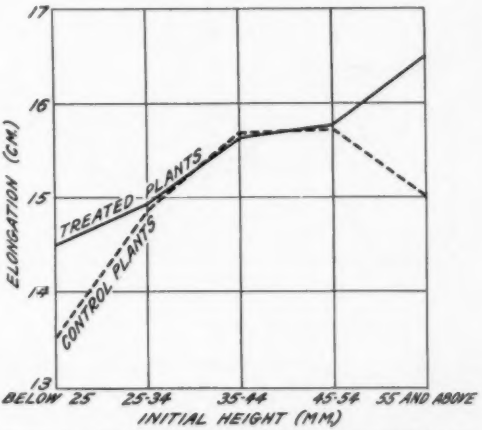

FIGURE 3.—Elongation of growing maize plants treated with a current of 10^{-9} amperes at night, experiment 128. Graph of data given in Table 5, showing the relation of elongation to initial height under treated and control conditions

Experiments 128 and 129 were analyzed further by sorting the individual plant measurements into groups corresponding to the initial height, and comparing the mean elongation of the treated plants of each group with the mean of the control plants having the same initial height.

In experiment 128 the measurements were sorted into five groups according to initial height. The class range was 10 mm., except that all plants with initial height below 24 mm. were combined, as were those above 54 mm. Fortunately, the numbers of treated and control in the several groups were nearly equal. The comparison is shown in Table 5 and Figure 3.

With plants ranging from 0 to 90 mm. in height and receiving the charge from a network only about 60 mm. above the tallest plant, it might be urged that some plants would be receiving too much and others too little current. If this were the case the agreement of the general mean of the treated with that of the control might result from combining plants that were stimulated with those that were retarded.

TABLE 5.—*Elongation of growing maize plants treated with a current of 10^{-9} amperes at night, experiment 128*

[Plants grouped according to initial heights. Treated plants compared with the control ones of the same group]

Initial height (mm.)	Treated		Control		Elongation difference (treated—control)	Difference ÷probable error
	Number of plants	Mean elongation (cm.)	Number of plants	Mean elongation (cm.)		
0–24	6	14.50	6	13.50	1.00	
25–34	45	14.93±0.21	40	14.85±0.23	.08±0.31	0.26
35–44	194	15.64± .08	195	15.68± .08	−.04± .11	.36
45–54	41	15.78± .15	40	15.75± .23	.03± .27	.11
55–90	2	16.50	4	15.00	−1.50	

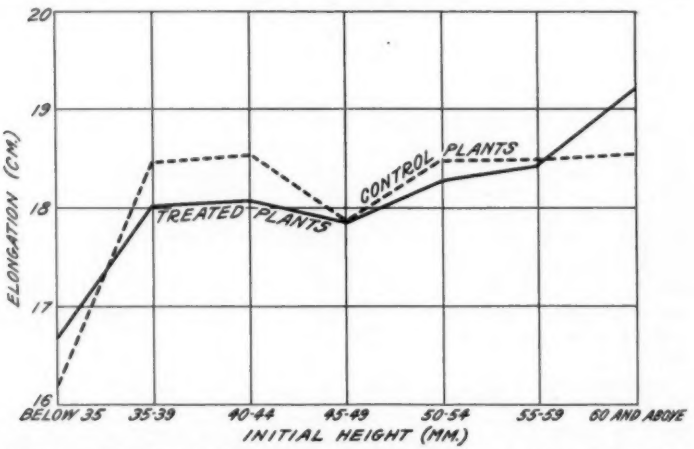

FIGURE 4.—Elongation of growing maize plants treated with a current of 10^{-9} amperes at night, experiment 129. Graph of data given in Table 6, showing the relation of elongation to initial height under treated and control conditions

With plants standing close together, as in this experiment, it seems certain that a great preponderance of the charged ions would pass to the taller plants. The close agreement of mean elongation in treated and control for all initial-height classes that are adequately represented fails to show any disturbing effect.

If the increased charge received by the taller plants was deleterious, the treatment should decrease the variability of elongation by permitting the shorter plants to overtake the taller ones in which growth is retarded. If, on the contrary, the treatment was so light as to affect the taller plants only, the variability should be increased through the increased growth of the taller plants. The standard deviation of elongation for the 288 treated plants was 1.72 cm., and that of the 285 control plants was 1.76, a difference of 0.04 cm., with a probable error of 0.07. Thus the comparison of variability gives no evidence that the treatment produced any measurable effect.

A similar analysis of experiment 129 is shown in Table 6 and Figure 4. The class range was 5 mm., and there were 7 classes. In none of the classes is the difference in elongation significant.

TABLE 6.—*Elongation of maize plants treated with a current of 10^{-9} amperes at night, experiment 129*

[Plants grouped according to initial heights. Treated plants compared with control ones of the same group]

Initial height (mm.)	Treated		Control		Elongation difference (treated − control)	Difference ÷ probable error
	Number of plants	Mean elongation (cm.)	Number of plants	Mean elongation (cm.)		
Below 35	9	16. 67±0. 85	10	16. 20±0. 70	0. 47±1. 10	0. 42
35–39	12	18. 00± . 38	11	18. 45± . 33	−. 45± . 50	. 90
40–44	26	18. 04± . 30	21	18. 52± . 48	−. 48± . 57	. 84
45–49	62	17. 84± . 16	66	17. 85± . 16	−. 01± . 23	. 04
50–54	88	18. 27± . 15	74	18. 43± . 16	−. 16± . 22	. 73
55–59	50	18. 42± . 17	55	18. 47± . 15	−. 05± . 23	. 22
60 and above	36	19. 22± . 21	48	18. 52± . 17	. 70± . 27	2. 59

The standard deviation of the 283 treated plants was 2.107 cm., and that of the 285 control plants was 2.131 cm., a difference of 0.024 cm., with a probable error of 0.08.

If there is any particular intensity of current that increases growth it might be expected that a very much stronger current would be detrimental. An experiment, therefore, was tried in every way similar to experiments 128 and 129 except that the current was increased to the maximum possible without sparking. The plants in this experiment, No. 130, received about 75×10^{-9} amperes each, or seventy-five times the intensity of the current in experiments 128 and 129.

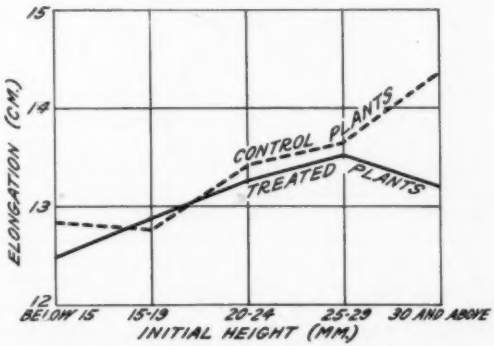

FIGURE 5.—Elongation of growing maize plants treated with a current of 75×10^{-9} amperes at night, experiment 130. Graph of data given in Table 7, showing the relation of elongation to initial height under treated and control conditions

A comparison of the mean values is given in the last line of Table 4. The results parallel those of experiments 128 and 129. Treated and control plants made practically the same elongation, and the uniformity of the experiment was such that a difference of 3 per cent would have been obvious. The standard deviation of the 282 treated plants was 1.66 cm., and that of the 284 control ones 1.79 cm. The smaller standard deviation of the treated plants should follow if the tallest plants were retarded, but the difference of 0.13 cm. is not significant.

The individual plant measurements were sorted on initial height as in experiment 129. The mean elongation of plants in the several initial-height classes are given in Table 7 and shown graphically in Figure 5. The agreement between treated and control is very close except in the group with the largest initial height. In this comparison of 30 treated plants with 28 control plants the means of the

control exceeded the treated by 3.5 times the probable error of the difference ($t=2.3$ and $P=$ about 0.03). Although a difference of this magnitude would be expected once in about thirty-five times, it suggests that the very tallest plants in the experiment may have been retarded in their growth. The five treated plants with the largest initial height had an elongation of 12 cm. The five largest control plants had a mean of 14.6 cm., and a difference of 2.6 ($t=2.3$, and $P=$ about 0.7).

TABLE 7.—*Elongation of growing maize plants treated with a current of* 75×10^{-4} *amperes at night, experiment 130*

[Plants grouped according to initial heights. Treated plants compared with control ones of the same group]

Initial height (mm.)	Treated		Control		Elongation difference (treated − control	Difference ÷ probable error
	Number of plants	Mean elongation (cm.)	Number of plants	Mean elongation (cm.)		
Below 15	23	12.48±0.28	17	12.82±0.41	−0.34±0.49	0.69
15–19	54	12.83± .15	65	12.78± .13	.05± .20	.25
20–24	96	13.26± .12	95	13.41± .12	−.15± .16	.94
25–29	79	13.54± .12	79	13.68± .12	−.14± .16	.87
30 and above	30	13.20± .23	28	14.32± .22	−1.12± .32	3.50

DISCUSSION

It is doubtless permissible to assume that, theoretically at least, all environmental changes will be found associated with changes in the growth rate if comparisons can be made with sufficient accuracy. In biological work, however, to measure the effect of a single factor with a probable error of 1 per cent represents a rather high degree of accuracy. It is quite possible to detect much smaller differences, but the difficulty is to associate these small differences with the factor under investigation.

The mean elongation of seedlings in a single box is so sensitive that unless great care is exercised uncontrolled and apparently insignificant differences in preparation will cause significant differences in the box means. Little difficulty was experienced in obtaining uniform behavior within the box. The variance of the row means, taken in either direction, was always of a magnitude to be expected from the variance of the individual measurements. Nevertheless it was only in the later experiments, where the precautions for uniformity of treatment were carried to what seems an absurd length, that four untreated boxes could be prepared in which the variance of box means was not significantly larger than the individual variance within the box. The negligibility of the electrical treatment is well shown by the fact that, although differences in the pine boxes that were of the same dimension and apparently alike were associated with different rates of growth, no effect of electrical treatment could be detected.

The 1927 and 1928 experiments appear to demonstrate that currents of the order of 10^{-9} amperes per plant applied during the night do not change the growth rate of maize seedlings.

The permutations of plant species, intensity of current, and time of application are very numerous, and the labor necessary to make

an adequate test of any particular combination is not small. In view of these considerations and the impracticability of utilizing any small increases that might be obtained by this means, further investigation of such permutations is not contemplated.

Great difficulty was experienced in providing an environment sufficiently uniform to eliminate significant differences in the rate of growth of cultures given the same treatment. This led to a demonstration that in many instances the association between treatment and a changed growth rate was not in the nature of cause and effect, and indicates that in experiments of this nature caution should be exercised in accepting conclusions based on the statistical significance of one type of experiment.

SUMMARY

The effect of electric currents on the growth of plants was tested by growing seedlings in small boxes in the laboratory. Current was supplied by suspending a charged network above the plants. Control boxes were treated in the same way except that the network was not charged.

Preliminary experiments with barley and maize, with a series of different intensities of current, and with the current applied for the entire period or restricted to the day or the night, indicated that maize treated at night with a current intensity of 10^{-9} amperes per plant gave most promise of positive results.

In the early experiments the measurement used was the height of the seedlings at the end of the experiment. In later experiments elongation of seedlings during the period of treatment was substituted for height. Elongation proved to be a more sensitive measure than height, since it eliminated a large part of the variation due to differences in the time of germination.

Slight differences in exposure due to the position of the boxes were found to cause differences in the rate of growth that masked any possible effect of the current or led to erroneous results. A more uniform exposure of treated and control boxes was provided by placing the entire experiment on a revolving table with the boxes symmetrically disposed about the center.

In the later experiments two boxes were subjected to treatment and two were grown as controls. Significant differences between the two treated or the two controls were taken as indicating the existence of uncontrolled factors that were differentiating the boxes. Differences of this nature were so common and of such magnitude as to destroy confidence in the results of any single experiment, although the statistical significance of the difference between treated and control might not be questioned.

A series of experiments, aimed to determine the nature of the disturbing factors that were causing significant differences in the growth of seedlings in boxes that were treated alike, was only partially successful. Although the boxes were of the same dimensions and all were made of the same kind of lumber, it was found that certain boxes were consistently associated with a slower growth of the seedlings. However, differences in the boxes did not account for all the observed differences in growth between similarly treated boxes.

By exercising extreme care in the preparation and planting of the boxes, and by planting more than the four boxes needed for an experi-

ment and selecting the four boxes showing the greatest uniformity, it was possible eventually to obtain a satisfactory degree of uniformity. Two preliminary series were run in which all four boxes were without treatment, and in no instance did the mean elongation of any box depart significantly from the mean of the experiment.

With similar precautions four experiments were run in which two of the boxes were subjected to a charge from the overhead network that induced a total discharge equal to 10^{-9} amperes per plant. In none of these experiments was there a measurable difference between treated and control in either weight or elongation. In the most uniform experiment a difference as small as 2 per cent of mean elongation would have been detected with practical certainty. In two of the four experiments the mean of the treated exceeded the control, and in two the reverse was the case.

The possibility that some plants received an excess of current that retarded their growth, while others were stimulated, was investigated by comparing the standard deviations of the treated and the control. There was no significant difference in the variability of the two series.

Three of the most satisfactory experiments were analyzed further by sorting the measurements of elongation into groups based on the initial size of the plant and restricting the comparison of the elongation of treated and control to plants of the same initial height. In no instance was there a significant difference. Since tall plants would receive more current than short ones, this constitutes, in effect, a test of the effect of currents both stronger and weaker than the prescribed current. While this method restricts the number of plants in any particular comparison and makes it impossible to detect small differences, the results may be taken as proving that under the conditions of these experiments no significant response is to be expected by altering the intensity of the treatment.

To test further the possibility of a detrimental effect of excessive currents, an experiment was tried with the maximum current possible without sparking. This was about 75 times the current used in the previous experiments. Even this excessive current did not produce any significant change in the rate of growth, though a difference of 3 per cent could have been detected under the conditions of the experiment.

A CRITERION OF THE DIFFERENTIATION OF VARIETIES OR OF EXPERIMENTAL AREAS WITH RESPECT TO THEIR CAPACITY TO PRODUCE SEEDLING STANDS OF COTTON [1]

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INTRODUCTION

Viability of seed has long been recognized as a factor of such importance as to justify the establishment of special laboratories where official tests under standard conditions may be conducted. The assumption is tacitly made that the germination rates as determined in the laboratory will be essentially comparable with those found in the field, or at least that various samples will stand in the same relative order in their germination in the field as in the laboratory.

In recent years there has been a tendency to question whether the laboratory test is an adequate indication of the results to be expected in the field. Both Whitcomb (*27*) [2] and Wright (*28*) have found large discrepancies between the germination rates of seed tested in the laboratory and the stand produced in the field.

While tests under controlled conditions doubtless give reasonably comparable and trustworthy results for different samples, especially when the differences are substantially larger than the probable errors of random sampling, it is not at all evident that varieties or samples planted under the conditions of practical agriculture will invariably stand in the relation to one another indicated by the results of laboratory trials. Certainly higher germination rates can not in general be expected in the field than in the laboratory.

Presumably many factors that do not affect percentage germination rate in the laboratory may influence seedling stand in the field. In the field the seed resumes its development under highly varying physical and chemical conditions and moisture content of the soil. In the production of seedling stand in the field, the capacity of the seedling for forcing its way through the soil and for withstanding the attacks of disease may be of great importance.

In earlier papers a criterion of the influence of extremely localized conditions on seedling stand (*12*) was considered and the applicability of Pearson's equivalent-probability correlation coefficient to the measurement of the relationship between the mortality of the seedlings of the same hill (*18*) was indicated. In the present paper two problems are to be considered: (1) That of the existence of a differential germination rate, or rather seedling-establishment rate, in the case of several pairs of varieties of cotton as grown under the conditions of irrigation agriculture in the arid Southwest; (2) that of the existence of differentiation of experimental areas with respect to their capacity for the production of seedling stand.

[1] Received for publication Jan. 2, 1929; issued May, 1929.

[2] Reference is made by number (italic) to "Literature cited," p. 619.

It might seem on first consideration that all that is required in the comparison of the germination rates of two varieties, or in two experimental areas, say x and y, is the determination (1) of the percentage of germination of the two varieties, or in the two areas, and the probable errors of these individual percentages and of the probable errors of the differences between them, or (2) of the mean numbers of seedlings germinating and the probable error of the mean number of seedlings germinating per unit area and the probable errors of these individual means and the probable error of the difference between these means in the event that a large number of tests of the same size has been made in the two cases. A comparison of the differences in percentage seedling stand in the one case or of mean seedling stand per unit area in the other, with due regard to the probable error of the differences as computed from the probable errors of the constants of the two varieties (taking into account the correlation between them when necessary), might seem to afford a sufficient basis for comparison.

This is not necessarily the case. An earlier study (*12*) showed that when seedling stand in the field is the basis of comparison the form of the frequency distribution of number of seedlings per hill may differ widely from experiment to experiment, and possibly from variety to variety. Thus it is quite possible that when grown in the field two varieties might have the same average germination rate but differ in the nature of the distribution of number of seedlings per hill when the planting rate is constant.

In such cases the problem is to determine whether the two distributions may be regarded as essentially identical, or, more precisely, whether they may be considered to be identical within the limits of the probable errors of random sampling. In essence the problem as stated in biometric terms reduces to the determination of the probability that the two distributions, representing two different varieties grown under as nearly as possible the same field conditions, may be regarded as samples drawn at random from the same population. If they can be so regarded, as far as the distribution of number of seedlings produced per unit area in a series of such tests shows, it is necessary to conclude that, however much the two varieties may be differentiated with respect to other morphological or physiological characters, they can not be considered to differ essentially in their capacity for the production of seedling stand. If, on the other hand, they can not be reasonably assumed to have been drawn from the same population, they can not be regarded as identical in their germination behavior under the given set of field conditions. Similar reasoning applies to the comparison of two different experimental areas or of two different planting methods when identical seed is employed.

The first step toward the solution of such problems was taken by Pearson (*24*), whose χ^2 criterion has long found application not merely in the testing of the goodness of fit of graduations of frequency distributions, but also in the investigation of various problems of the distribution of disease, in the testing of the goodness of fit of Mendelian ratios (*4*), and more recently in testing the departures of the frequencies of seedlings per hill from a point binomial distribution (*12*).

In 1911 Pearson extended his theory (*25*) to include cases in which it is needful to ascertain whether two independent distributions of frequency are really samples from the same population.

This is the criterion particularly emphasized in the present investigation. The problem in hand is to determine whether two distributions of frequencies of number of seedlings per hill are so nearly identical that they may be reasonably assumed to represent the same population, or whether they are so different that the odds against their having been drawn from one and the same population are overwhelmingly large.

In Pearson's proof the assumption is properly made that the two distributions of frequency are wholly independent. This condition is fully realized in the comparison of the seedling stands of the same variety as grown on two different plots or under different cultural conditions. Since the various series of cotton varieties here compared were grown pair by pair on very small and closely associated plots, it is probable that some correlation will be found between the seedling stand of adjacent hills of the different varieties. This seems probable (1) from the results that have been found for the influence of field heterogeneity on a wide range of crop characters, including both the existence of heterogeneity in a given year (*5*, *6*, *8*) and the permanence of the differentiation of the plots of an experimental field from year to year (*19*, *20*), and (2) from the results already obtained for cotton. These latter have shown that there is a fairly high correlation between closely associated plants for osmotic concentration, specific electrical conductivity, and hydrogen-ion concentration of the leaf-tissue fluids (*17*) and for chloride (*16*) and sulphate (*13*) contents. The regressions of certain of these plant characters and soil properties have been shown to be practically linear (*11*). These evidences of the influence of soil heterogeneity on the characteristics of the cotton plant have been further supported by the demonstration of rather large correlations between the electrical resistance of the saturated soil mass as measured by the soil bridge (*2*) and the physicochemical properties of the leaf-tissue fluids of the cotton plant (*7*).

For the seedling itself there are available not merely the results cited above (*12*, *18*) for the influence of highly localized conditions on seedling stand, but also some evidence of a measurable correlation between soil salinity and seedling stand (*10*).

While some correlation between the seedling stands of adjacent areas doubtless exists, it is probable that this correlation (the actual magnitude of which is now under investigation) will not be such as to invalidate the use of Pearson's criterion for the purpose for which it is here recommended.

Let x and y be two variables to be compared on the basis of their respective frequency distributions, which have the same number of classes, s. The number of individuals of x and y in any class will be n_x and n_y. The totals of the two distributions will be $S\ (n_x) = N_x$, and $S\ (n_y) = N_y$.

In the present notation Pearson's formula is

$$\sum_{1}^{s}\left\{\frac{N_x N_y\left(\frac{n_x}{N_x}-\frac{n_y}{N_y}\right)^2}{n_x+n_y}\right\}=\chi^2 \qquad (1)$$

This is a general formula, applicable whatever the value of N_x and N_y. In these experiments $N_x = N_y$ and after some reduction the equation becomes

$$\sum_{1}^{s} \left\{ \frac{(n_x - n_y)^2}{n_x + n_y} \right\} = \chi^2 \qquad (2)$$

thus greatly simplifying the arithmetical routine.

The method of calculation of the double χ^2 criterion for cases in which the number of hills is the same in the two series is illustrated in Table 1, which represents the data given in Table 2 for Pima Egyptian and Acala upland cotton germinated under light irrigation.

TABLE 1.—*Calculation of double χ^2 for Pima and Acala cottons planted with light irrigation at the United States Field Station, Sacaton, Ariz., 1926*

Number of seedlings per hill	Pima	Acala	Pima− Acala	Pima+ Acala	$\frac{(\text{Pima}-\text{Acala})^2}{\text{Pima}+\text{Acala}}$
0	65	136	−71	201	25.0796
1	42	180	−138	222	85.7838
2	67	308	−241	375	154.8827
3	182	452	−270	634	114.9842
4	343	520	−177	863	36.3024
5	692	386	+306	1,078	86.8608
6	737	146	+591	883	395.5617
Total	2,128	2,128	0	4,256	899.4552

TABLE 2.—*Frequency distribution of number of seedlings per hill in Pima, Egyptian, and Acala upland cottons as grown at Sacaton, Ariz., in experiment 1/26 (1926)*

Number of seedlings per hill	Normal irrigation		Light irrigation	
	Pima	Acala	Pima	Acala
0	363	365	65	136
1	73	145	42	180
2	83	234	67	308
3	144	341	182	452
4	302	470	343	520
5	510	399	692	386
6	653	174	737	146
Total	2,128	2,128	2,128	2,128

From the values of χ^2 thus determined the values of P may be computed from Elderton's table (*3, 26*).

While the present paper emphasizes one method of attack on the problem of the existence of statistically significant differences in the capacity of the varieties for the establishment of seedling stand, it must be understood that there are various possible ways of making the necessary comparisons.

The varieties may be compared with respect to any particular frequency class that is of special interest. Such classes are those with zero or a very low number of seedlings per hill and those with a very large number of seedlings per hill. In some cases it may be desirable to compare the germination rates on the basis of but two or three classes secured by the combination of a number of others.

A number of methods are not merely possible but are desirable. The reason that one method is especially stressed here lies solely in the facts that it is novel for studies of this kind and that it makes possible the most rigid and detailed comparisons of the frequency distribution as a whole which is yet available.

MATERIALS

With the exception of one experiment, the necessary data are given in Tables 3 to 10 of an earlier paper (*12*), in which they were considered merely from the standpoint of their deviation from a random distribution. The one series of data not available in a previous United States Department of Agriculture publication represents an experiment of 1926 (experiment 1/26) made to compare Pima Egyptian and Acala upland cottons as grown under light and heavy irrigation. The frequencies of the number of seedlings per hill in this experiment are shown in Table 2. Here the frequencies under the heading "Normal irrigation" represent the stand secured on plots C 3–16 and C 3–17, while those under the heading "Light irrigation" represent the stands secured on plots C 3–18 and C 3–19, all of the United States Field Station at Sacaton, Ariz. These four plots were immediately adjoining and were apparently identical except for differences in their immediate experimental treatments.

Since in the two light-irrigation plots the object was to apply the minimum quantity of water necessary to secure the germination of seed, the ridge method of planting was adopted. In the normal-irrigation series the flooding method was used. Since the present purpose is merely to consider the methods of comparison of the seedling stand produced by two different cultural methods, the details of these experiments need not be discussed further.

The comparisons involve three types of cotton, sea island, Egyptian, and upland. With regard to these three types it is sufficient to note that sea island and Egyptian are similar in many respects and that both are usually referred to the species *Gossypium barbadense*. Upland cotton belongs to another species (*G. hirsutum*), which differs from *G. barbadense* in many important morphological characters. Some of these have been enumerated by Kearney (*22, p. 4–6*).

There are also differences in the physicochemical properties of the tissue fluids of the types, especially in osmotic concentration, specific electrical conductivity, and hydrogen-ion concentration (*15, 17*), and chloride (*16*) and sulphate (*13*) contents, with respect to which anions absorption has been shown to be differential (*9, 14*). The differentiation of the Pima Egyptian and Acala upland varieties with respect to their water relations has recently been discussed by Hawkins (*21*).

Two of the varieties considered require discussion in greater detail. These are smooth-seeded Pima, a variant of the normal Pima type, and okra-leaved Acala, a possible mutant of the Acala type. Comparisons of these two types with the parent forms are of special interest.

T. H. Kearney states that the smooth-seeded family of Pima Egyptian is derived from an individual selected in 1920 by Walter F. Gilpin in a field of the ordinary, rather fuzzy-seeded stock of the Pima variety. This individual had nearly naked seeds and proved to be homozygous for this character, its descendants having been

uniformly smooth seeded. Investigations by Kearney and Harrison (*23*) have shown the smooth-seeded character of the seed coat to be dominant to the fuzzy-seeded condition in the Pima variety. Smooth seeds are a great advantage in long-staple cottons, as ginning on the roller gin is much more rapid with a smooth-seeded than with a fuzzy-seeded type. Unfortunately, this family has been found to be inferior in other respects, having smaller bolls and shorter and weaker fiber than the ordinary Pima.

Okra-leaved Acala is a form originally isolated and developed by H. Gregory McKeever, of the Office of Cotton, Rubber, and Other Tropical Plants. It is characterized by deeply divided leaves.

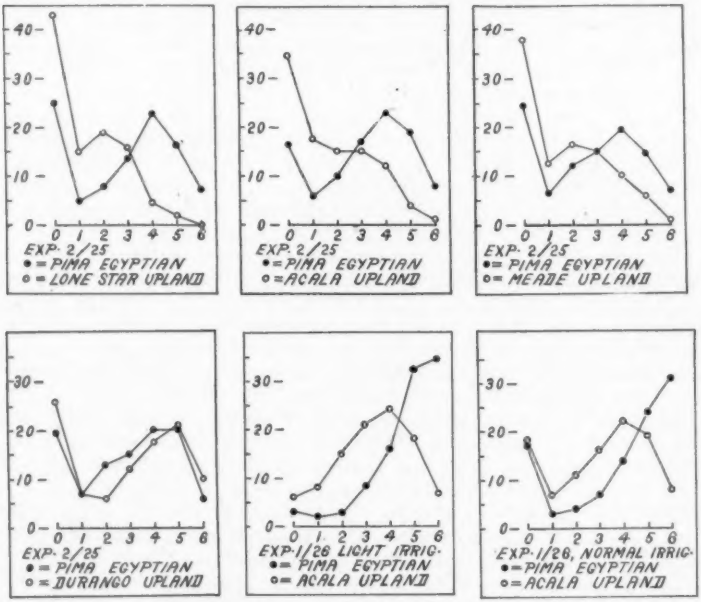

FIGURE 1.—Comparison of frequency distributions of numbers of seedlings per hill in Pima Egyptian and various upland varieties of cotton

Recently it attracted considerable attention as a commercial possibility in some regions of the Southwest. It has been briefly described and partially illustrated by Cook and Doyle (*1, p. 23–24, pl. 11*) under the less characteristic term of a "narrow-leaf selection of Acala cotton."

RESULTS

The differences between the several frequency distributions of numbers of seedlings per hill are represented graphically in the several comparison diagrams. Since these frequency distributions represent different numbers of hills in the several experiments, all have been reduced to a percentage basis. In each of Figures 1 to 7 the axis of abscissae represents number of seedlings per hill and the axis of ordinates represents the percentage frequencies of the several classes.

Table 3 gives the final results of the χ^2 test of the significance of the differentiation of the distributions of the number of seedlings per hill in the varieties compared.

The only criterion that will be treated here in comparison with the χ^2 test of the differentiation of the two distributions as a whole is the difference in the mean numbers of seedlings per hill. The differences between the means of the numbers of seedlings per hill in the pairs of varieties that may be compared and the values of the χ^2 and P criteria are shown in Table 3.

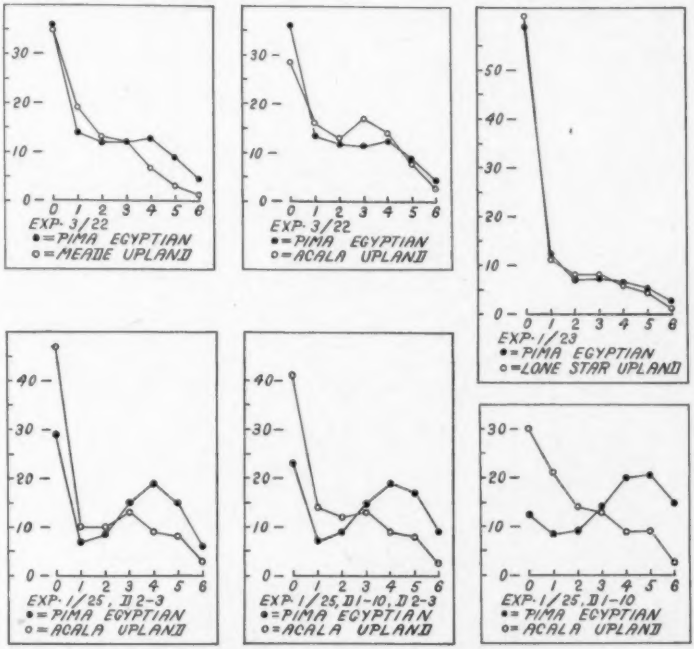

FIGURE 2.—Comparison of frequency distributions of numbers of seedlings per hill in Pima Egyptian and various upland varieties of cotton

COMPARISON OF STANDS PRODUCED BY DIFFERENT VARIETIES

A full understanding of the results derived from the means and from the χ^2 and P criteria necessitates a consideration of the frequency distributions for the several pairs of varieties compared.

In Table 3 the numbers of the experiments and the comparisons made, together with references to the tables giving the original frequency distributions for number of seedlings per hill, are indicated in column 1. The number of hills of each of the two varieties is given in column 2. The number of the figure in which the distributions of the two varieties are compared is entered in column 3. The means and probable errors of the number of seedlings per hill for the two series compared are given as (1) and (2) in columns 4 and 5, the order being that of the varieties given in column 1. The differences

between the mean numbers of seedlings per hill in the two varieties, together with their probable errors, are given in column 6. The differences are derived thus: Constant for second variety less constant for first variety. This fact must be borne in mind in interpreting the differences. The ratios of the differences to their prob-

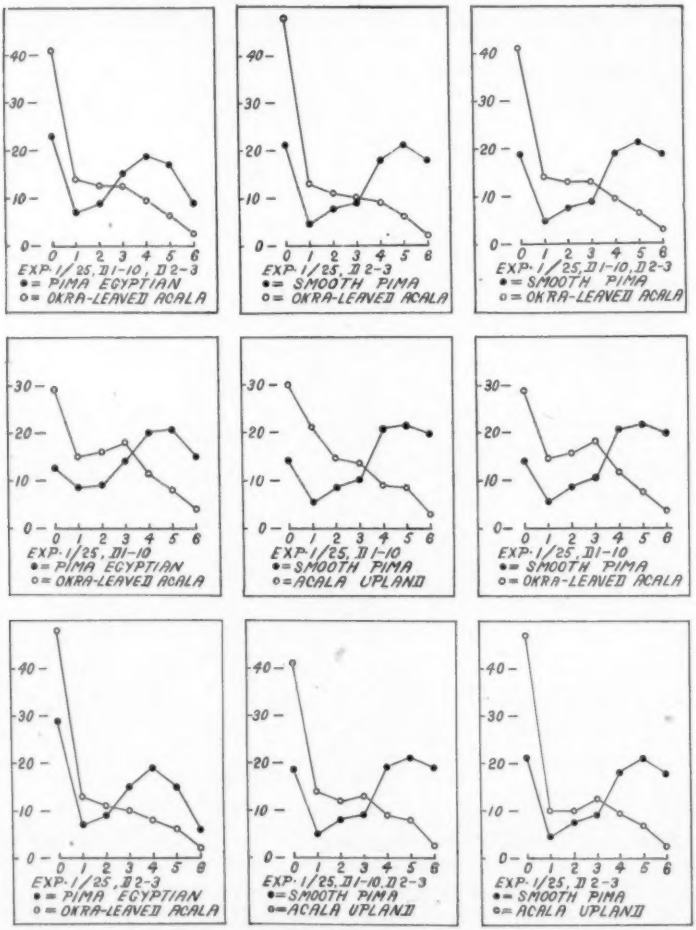
EXP. 1/25, D 1-10, D 2-3
● = PIMA EGYPTIAN
○ = OKRA-LEAVED ACALA
EXP. 1/25, D 2-3
● = SMOOTH PIMA
○ = OKRA-LEAVED ACALA
EXP. 1/25, D 1-10, D 2-3
● = SMOOTH PIMA
○ = OKRA-LEAVED ACALA
EXP. 1/25, D 1-10
● = PIMA EGYPTIAN
○ = OKRA-LEAVED ACALA
EXP. 1/25, D 1-10
● = SMOOTH PIMA
○ = ACALA UPLAND
EXP. 1/25, D 1-10
● = SMOOTH PIMA
○ = OKRA-LEAVED ACALA
EXP. 1/25, D 2-3
● = PIMA EGYPTIAN
○ = OKRA-LEAVED ACALA
EXP. 1/25, D 1-10, D 2-3
● = SMOOTH PIMA
○ = ACALA UPLAND
EXP. 1/25, D 2-3
● = SMOOTH PIMA
○ = ACALA UPLAND

FIGURE 3.—Comparison of frequency distributions of numbers of seedlings per hill in normal and smooth-seeded Pima Egyptian and in okra-leaved and normal Acala upland varieties of cotton

able errors are given in column 7. The values of χ^2 and of P are presented in columns 8 and 9.

The distribution of number of seedlings per hill in Pima Egyptian cotton as compared with that in a number of upland varieties is first to be considered. The normal Pima and the normal upland varieties are represented in Figures 1 and 2. Further comparisons of the

normal Pima with the okra-leaved Acala upland cotton and of the smooth-seeded Pima with the normal Acala and with the okra-leaved Acala cotton appear in Figure 3.

An inspection of Figures 1 and 2 shows that, in general, stands of Pima Egyptian cotton are characterized by a lower frequency of hills with no seedlings or with few seedlings and by a materially higher percentage of hills with more seedlings than those of the normal upland varieties with which they are compared.

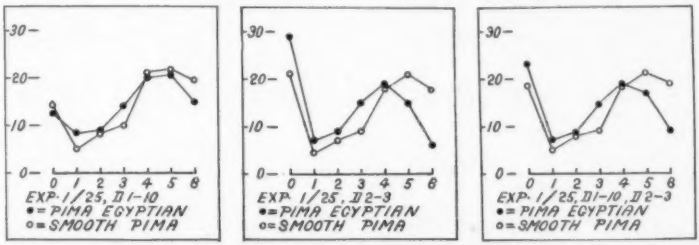

FIGURE 4.—Comparison of frequency distributions of numbers of seedlings per hill in normal and smooth-seeded Pima Egyptian cottons

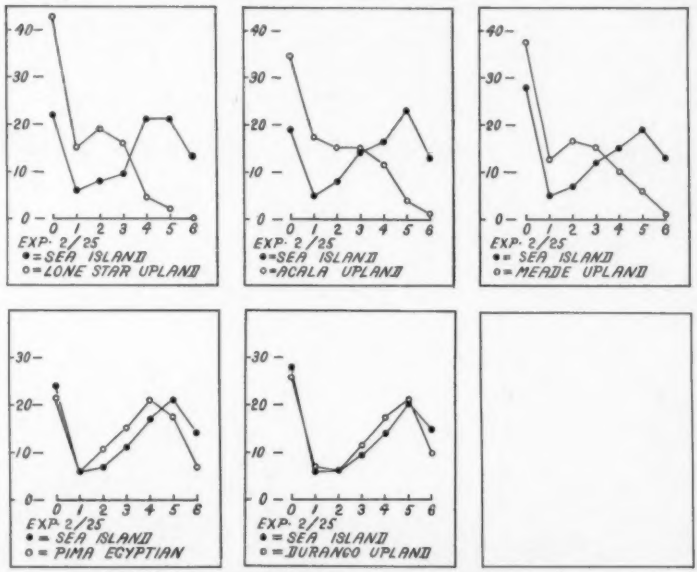

FIGURE 5.—Comparison of frequency distributions of numbers of seedlings per hill in sea-island and various upland varieties of cotton

A summary of the final results of the two comparisons from Table 3 gives the ratios of the differences in the means to the probable errors of these differences and the values of P. Here the signs of the differences between the means (taken to be positive when the mean of the second variety is larger than that of the first variety mentioned) are prefixed to the ratios of the differences to their probable errors. The results are given in Table 4.

TABLE 3.—*Criteria of differences in the capacities of varieties of sea-island, Pima Egyptian, and upland cottons for the production of seedling stand*

Experiment and variety [a]	Number of hills	Figure No.	Mean (1)	Mean (2)	Difference (2)−(1)	Diff. / $E_{diff.}$	x^2	P
Experiment 3/22, 1922, plots E 3–1, E 3–2, E 3–3, Table 3:								
Pima (Egyptian) and Acala (upland)	1,440	2	1.9750±0.0345	2.0785±0.0320	+0.1035±0.0470	2.20	37.5960	0.000016
Pima (Egyptian) and Meade (upland)	1,440	2	1.9750± .0345	1.3118± .0275	− .6632± .0441	15.05	100.0901	.000000
Acala (upland) and Meade (upland)	1,440	6	2.0785± .0320	1.3118± .0275	− .7667± .0422	18.18	146.7670	.000000
Experiment 1/23, 1923, plots D 1–10, D 1–11, Table 4:								
Pima (Egyptian) and Lone Star (upland)	1,440	2	1.1674±.0308	1.0500± .0285	− .1174± .0420	2.79	9.1317	.167134
Experiment 1/25, 1925, plot D 2–3, Table 5:								
Pima (Egyptian) and smooth-seeded Pima	720	4	2.5750± .0513	3.3403± .0547	+ .7653± .0750	10.20	74.0113	.000000
Pima (Egyptian) and Acala (upland)	720	2	2.5750± .0513	1.5944± .0464	− .9806± .0692	14.17	90.0488	.000000
Pima (Egyptian) and okra-leaved Acala	720	3	2.5750± .0513	1.4556± .0445	−1.1194± .0679	16.49	189.7403	.000000
Smooth-seeded Pima and Acala (upland)	720	3	3.3403± .0547	1.5944± .0464	−1.7458± .0718	24.33	244.4199	.000000
Smooth-seeded Pima and okra-leaved Acala	720	3	3.3403± .0547	1.4556± .0445	−1.8847± .0705	26.72	281.4961	.000000
Acala (upland) and okra-leaved Acala	720	7	1.5944± .0464	1.4556± .0445	− .1389± .0643	2.16	7.1228	.310686
Experiment 1/25, 1925, plot D 1–10, Table 6:								
Pima (Egyptian) and smooth-seeded Pima	400	4	3.4150± .0655	3.6100± .0676	+ .1950± .0942	2.07	7.7719	.256977
Pima (Egyptian) and Acala (upland)	400	2	3.4150± .0655	1.8700± .0596	−1.5450± .0886	17.44	124.4109	.000000
Pima (Egyptian) and okra-leaved Acala	400	3	3.4150± .0655	2.0550± .0603	−1.3600± .0891	15.27	101.1199	.000000
Smooth-seeded Pima and Acala (upland)	400	3	3.6100± .0676	1.8700± .0596	−1.7400± .0902	19.30	141.6055	.000000
Smooth-seeded Pima and okra-leaved Acala	400	3	3.6100± .0676	2.0550± .0603	−1.5550± .0906	17.16	135.7256	.000000
Acala (upland) and okra-leaved Acala	400	7	1.8700± .0596	2.0550± .0603	+ .1850± .0848	2.18	9.3266	.157599
Experiment 1/25, 1925, plots D 1–10, D 2–3, Tables 5 and 6:								
Pima (Egyptian) and smooth-seeded Pima	1,120	4	2.8750± .0412	3.4366± .0427	+ .5616± .0594	9.46	65.2797	.000000
Pima (Egyptian) and Acala (upland)	1,120	2	2.8750± .0412	1.6928± .0368	−1.1821± .0552	21.40	202.8219	.000000
Pima (Egyptian) and okra-leaved Acala	1,120	3	2.8750± .0412	1.6696± .0363	−1.2054± .0549	21.95	212.7705	.000000
Smooth-seeded Pima and Acala (upland)	1,120	3	3.4366± .0427	1.6928± .0368	−1.7438± .0564	30.93	399.8523	.000000
Smooth-seeded Pima and okra-leaved Acala	1,120	3	3.4366± .0427	1.6696± .0363	−1.7670± .0561	31.52	412.3170	.000000
Acala (upland) and okra-leaved Acala	1,120	7	1.6928± .0368	1.6696± .0363	− .0232± .0516	.45	1.6630	.941912
Experiment 2/25, 1925, plot D 2–4, Tables 7–10:								
Pima (Egyptian) and Acala (upland)	240	1	3.1167± .0820	1.6958± .0710	−1.4208± .1085	13.10	73.3728	.000000
Pima (Egyptian) and Durango (upland)	240	1	2.9375± .0837	2.9125± .0938	− .0250± .1257	.20	11.8702	.065397
Pima (Egyptian) and Lone Star (upland)	240	1	2.8458± .0884	1.3000± .0602	−1.5458± .1059	14.46	109.5518	.000000
Pima (Egyptian) and Meade (upland)	240	1	2.7083± .0863	1.7125± .0740	− .9958± .1137	8.76	37.3771	.000025
Pima (Egyptian) and sea island	960	5	2.9021± .0427	3.0812± .0473	+ .1792± .0637	2.81	42.0833	.000000
Experiment 2/25, 1925, plot D 2–4, Tables 7–10:								
Sea island and Acala (upland)	240	5	3.2583± .0897	1.6958± .0710	−1.5625± .1144	13.66	88.4003	.000000
Sea island and Durango (upland)	240	5	2.9792± .0990	2.9125± .0938	− .0667± .1364	.49	3.7971	.703493
Sea island and Lone Star (upland)	240	5	3.1750± .0919	1.3000± .0602	−1.8750± .1098	17.07	135.7772	.000000
Sea island and Meade (upland)	240	5	2.9125± .0967	1.7125± .0740	−1.2000± .1217	9.86	62.8422	.000000
Sea island and Pima (Egyptian)	960	5	3.0812± .0473	2.9021± .0427	− .1792± .0637	2.81	42.0833	.000000

Experiment 2/25, 1925, plot D 2–4, Tables 7-10:								
Acala (upland) and Meade (upland)	240	6	1.6958± .0710	1.7125± .0740	+.0167± .1026	.16	3.6682	.720531
Acala (upland) and Durango (upland)	240	6	1.6958± .0710	2.9125± .0939	+1.2167± .1177	10.34	46.6392	.000000
Acala (upland) and Lone Star (upland)	240	6	1.6958± .0710	1.3000± .0602	+.3958± .0931	4.25	15.5332	.016789
Durango (upland) and Lone Star (upland)	240	6	2.9125± .0939	1.3000± .0602	−1.6125± .1115	14.46	114.2655	.000000
Durango (upland) and Meade (upland)	240	6	2.9125± .0939	1.7125± .0740	−1.2000± .1195	10.04	61.4956	.000000
Lone Star (upland) and Meade (upland)	240	6	1.3000± .0602	1.7125± .0740	−.4125± .0954	4.32	15.3907	.017716
Experiment 1/26, 1926, normal-irrigation plots C 3–16, C 3–17, Table 1:								
Pima (Egyptian) and Acala (upland)	2,128	1	3.9225± .0318	3.0804± .0278	−.8421± .0422	19.93	503.2831	.000000
Experiment 1/26, 1926, light-irrigation plots C 3–18, C 3–19, Table 1:								
Pima (Egyptian) and Acala (upland)	2,128	1	4.6880± .0213	3.3073± .0213	−1.3806± .0301	45.90	899.4553	.000000

* Table 1 refers to the present paper; other table numbers refer to Harris, Harrison, and Wadley (*12*).

An examination of the differences in the mean number of seedlings per hill shows that in 11 of the 12 comparisons the average number is higher in Pima Egyptian than in the upland varieties. In the one case in which the upland variety shows a higher mean number of seedlings (experiment 3/22, Pima and Acala), the difference is only

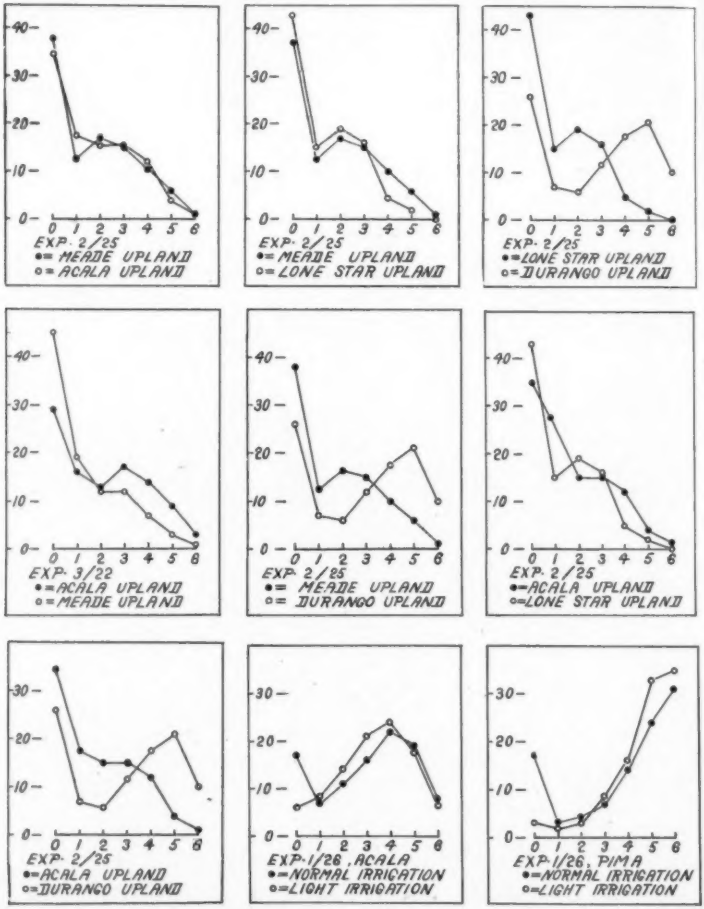

FIGURE 6.—Comparison of frequency distributions of numbers of seedlings per hill in upland varieties of cotton. Also comparisons of frequency distributions of numbers of seedlings per hill in Acala upland and Pima Egyptian cottons as grown under normal and light irrigation

2.2 times as large as its probable error. Of the 11 differences that indicate superior stands in the Pima variety, only 1 is less than 2.5 times as large as its probable error. The other differences range from 2.79 to 45.9 times as large as their probable errors.

This comparison between Pima Egyptian and the several typical upland varieties by means of the χ^2 criterion shows that in 8 of the

TABLE 4.—*Summary of comparisons of Pima Egyptian with typical upland cottons*

Experiment and varieties of cotton compared	Diff. / E diff.	*P*
Experiment 3/22:		
Pima and Acala (E 3-1 to E 3-3)	+2.20	0.000016
Pima and Meade (E 3-1 to E 3-3)	−15.05	.000000
Experiment 1/23:		
Pima and Lone Star (D 1-10 to D 1-11)	−2.79	.167134
Experiment 1/25:		
Pima and Acala (D 2-3)	−14.17	.000000
Pima and Acala (D 1-10)	−17.44	.000000
Pima and Acala (D 2-3 to D 1-10)	−21.40	.000000
Experiment 2/25:		
Pima and Acala (D 2-4)	−13.10	.000000
Pima and Durango (D 2-4)	−.20	.065397
Pima and Lone Star (D 2-4)	−14.46	.000000
Pima and Meade (D 2-4)	−8.76	.000025
Experiment 1/26:		
Pima and Acala (C 3-16 to C 3-17)	−19.93	.000000
Pima and Acala (C 3-18 to C 3-19)	−45.90	.000000

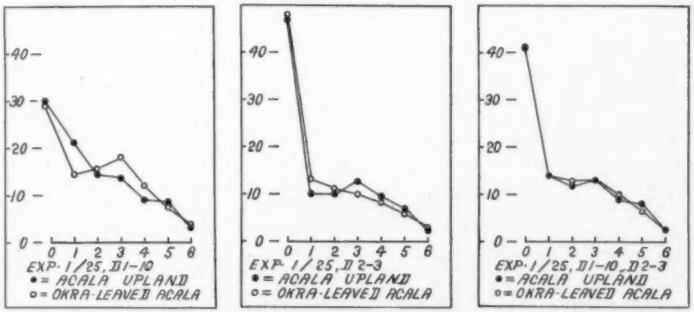

FIGURE 7.—Comparison of frequency distributions of numbers of seedlings per hill in normal and okra-leaved Acala upland cotton

12 cases the value of *P* indicates that the probabilities of the two distributions being identical within the limits of the probable errors of random sampling are less than one in a million. In the four cases in which significant figures occur in *P*, the chances of the differences between the two distributions having arisen through random sampling are very small indeed (0.000016 for Pima and Acala in experiment 3/22, 0.167134 for Pima and Lone Star in experiment 1/23, 0.000025 for Pima and Meade in experiment 2/25, and 0.065397 for Pima and Durango in experiment 2/25).

These results evidence in the strongest way the differentiation of Pima Egyptian cotton from the upland varieties with respect to its capacity for the production of seedling stand under the conditions of these experiments.

If these comparisons be extended to include those between the smooth-seeded variety of Pima and the normal and abnormal upland types and between normal Pima and the abnormal upland types, the results are those given in Table 5.

TABLE 5.—*Summary of comparisons of smooth-seeded Pima with normal and okra-leaved Acala cotton and of normal Pima with okra-leaved Acala cotton in experiment 1/25*

Varieties of cotton compared	$\frac{\text{Diff}}{\text{E diff}}$	P
Pima and okra-leaved Acala, (D 2-3)	−16.49	0.000000
Pima and okra-leaved Acala, (D 1-10)	−15.27	.000000
Pima and okra-leaved Acala, (D 2-3, D 1-10)	−21.95	.000000
Smooth-seeded Pima and Acala (D 2-3)	−24.33	.000000
Smooth-seeded Pima and Acala (D 1-10)	−19.30	.000000
Smooth-seeded Pima and Acala (D 2-3, D 1-10)	−30.93	.000000
Smooth-seeded Pima and okra-leaved Acala (D 2-3)	−26.72	.000000
Smooth-seeded Pima and okra-leaved Acala (D 1-10)	−17.16	.000000
Smooth-seeded Pima and okra-leaved Acala (D 2-3, D 1-10)	−31.52	.000000

The results in Table 5 show overwhelming evidence of the superiority of the normal and the smooth-seeded Pima Egyptian to the normal and the okra-leaved Acala for the establishment of seedling stands. In all cases the average number of seedlings produced is larger in Pima Egyptian than in Acala upland cotton. In all cases the significant figures of P lie beyond 0.000000.

Grouping these 9 comparisons with those given above for the normal Pima Egyptian as compared with normal upland varieties (Table 4), it appears that of the 21 comparisons only 1 shows a slightly larger average number of seedlings per hill in the upland type. In all other cases the results indicate a superiority of Egyptian cotton for the production of a larger mean number of seedlings per hill. The ratios of these differences to their probable errors vary from 2.79 to 45.9. The values of the χ^2 and P criteria indicate chances of less than one in a million of the two varieties compared being undifferentiated in only 4 of the 21 cases.

It is now desirable to consider the limited data available for a comparison of the smooth-seeded Pima with the normal type. The comparison is made in Figure 4.

While the two percentage frequency distributions are more nearly superimposed in the three panels of Figure 4 than in those discussed above, there is an apparent tendency for the smooth-seeded Pima to show slightly smaller percentages of hills with a small number of seedlings and relatively larger percentages for hills with the higher numbers of seedlings.

The positive differences between the means (Table 6) show that the smooth-seeded Pima gives higher seedling stands than the normal type. The values for the χ^2 criterion in this case show for one plot (D 1–10) chances of 26 in 100 of an insignificant difference, while for the other (D 2–3) and for the combination of the two (D 1–10 and D 2–3) the chances are less than one in a million of differences as large as those observed having arisen through random sampling. While the values of χ^2 are low as compared with those for some other comparisons of experiment 1/25, they seem to indicate that the smooth-seeded Pima variety has at least a slight superiority for the establishment of seedling stand. This problem should, however, receive further consideration on the basis of additional experimental data.

TABLE 6.—*Summary of comparisons of normal and smooth-seeded Pima Egyptian cotton in experiment 1/25*

Varieties of cotton compared	$\frac{\text{Diff.}}{E_{\text{diff.}}}$	P
Pima and smooth-seeded Pima (D 2–3)	+10.20	0.000000
Pima and smooth-seeded Pima (D 1–10)	+2.07	.256977
Pima and smooth-seeded Pima (D 2–3, D 1–10)	+9.46	.000000

The results of the comparisons that have been made of the germination of sea-island and of upland cotton may now be considered. These results appear in Figure 5. This figure shows comparisons between sea-island cotton and the upland cottons—Acala, Durango, Lone Star, and Meade.

TABLE 7.—*Summary of comparisons of sea-island with upland cottons in experiment 2/25*

Varieties of cotton compared	$\frac{\text{Diff.}}{E_{\text{diff}}}$	P
Sea island and Acala (D 2–4)	−13.66	0.000000
Sea island and Durango (D 2–4)	−.49	.703493
Sea island and Lone Star (D 2–4)	−17.07	.000000
Sea island and Meade (D 2–4)	−9.86	.000000

The results from experiment 2/25 are shown in Table 7. In three of these comparisons (those for Acala, Lone Star, and Meade upland cottons) the distributions show that sea-island cotton has a conspicuously smaller number of hills with no seedlings or with a small number of seedlings and a conspicuously larger number of hills with a larger number of seedlings. In these three cases the double χ^2 test shows that there is less than one chance in a million of the difference being due to random sampling, the significance as indicated by the values of χ^2 being especially marked for Lone Star and Acala. In the fourth case (the comparison involving Durango upland cotton) the difference is not so marked. In this case the double χ^2 test shows chances of 0.70, on the basis of a total probability of 1, of the differences being due to the probable errors of random sampling. It appears, therefore, that in three of the comparisons between sea-island and upland cotton as grown in 1925 the sea-island cotton shows distinct superiority over the upland type with respect to its capacity for the production of seedling stand. In a fourth comparison (with Durango), however, the difference is not significant.

Figure 5 contains one comparison of sea-island and Pima Egyptian cottons. The differences in the distributions are not large. The sea-island type has a slightly larger percentage of hills with no seedlings and a slightly larger number of hills with 5 or 6 seedlings than does the Pima Egyptian type. Hills with 2, 3, and 4 seedlings are more abundant in the Pima Egyptian type. The ratio of the difference to the probable error of the difference is +2.81, and P is 0.000000. The positive sign of the ratio of the difference between the two means to its probable error shows that in this experiment sea island produced a somewhat better stand than Pima Egyptian. While the difference between the distributions of number of seedlings per hill in Egyptian

and sea-island cotton is clearly significant, since the value of P shows that it could have been expected to arise through random sampling less than once in a million times, final conclusions as to the generality of differences between the seedling stands of these two types of cotton must be based on additional experimental data.

A comparision between the seedling stands of pairs of upland varieties is shown in Figure 6. The ratios of the differences in mean germination rate and the values of P are summarized in Table 8. These show great irregularities in sign and magnitude. This is to be expected, since the seven comparisons made involve four different upland varieties. Before any final conclusion can be drawn concerning the superiority or inferiority of any individual upland variety, much more extensive comparisions must be made.

TABLE 8.—*Summary of comparisons of various upland cottons*

Experiment and varieties of cotton compared	$\frac{\text{Diff.}}{E_{\text{diff.}}}$	P
Experiment 3/22:		
Acala and Meade	−18.18	0.000000
Experiment 2/25:		
Acala and Meade	+.16	.720531
Acala and Durango	+10.34	.000000
Acala and Lone Star	−4.25	.016789
Durango and Lone Star	−14.46	.000000
Durango and Meade	−10.04	.000000
Lone Star and Meade	+4.32	.017716

The differences between the distributions of the seedling stands of Acala upland cotton and the okra-leaved mutant type of Acala cotton in the one available experiment are shown in Figure 7. This experiment involved plantings on two different plots. The results are presented for the plots individually and in combination. To the eye the distributions seem essentially identical.

The differences (Table 9) in the means are not consistent in sign for the two plots, and could not safely be considered significant in comparison with their individual probable errors. The difference in the means for the experiment as a whole is certainly insignificant. The χ^2 criterion shows that chances of insignificance are 16, 31, and 94 in 100. It appears, therefore, that no difference can be asserted to exist between the capacities of the normal and the okra-leaved Acala cotton for the establishment of seedling stand.

TABLE 9.—*Summary of comparisons of normal and okra-leaved Acala cotton in experiment 1/25*

Varieties of cotton compared	$\frac{\text{Diff.}}{E_{\text{diff.}}}$	P
Acala and okra-leaved Acala D 2-3	−2.16	0.310686
Acala and okra-leaved Acala D 1-10	+2.18	.157599
Acala and okra-leaved Acala D 1-10 to D 2-3	−.45	.941912

COMPARISON OF STANDS PRODUCED UNDER DIFFERENT CONDITIONS

A comparison between the stands secured with normal and with light irrigation is shown in Figure 6. In both of the varieties used the number of hills that produced no seedlings was conspicuously higher when the plants were provided with normal irrigation at planting time than when they were planted by the ridge method and lightly irrigated. The differences for the other frequencies are not so conspicuous.

The four means to be compared may be obtained from Table 3, and are shown in Table 10.

TABLE 10.—*Comparison of mean seedling stands of cotton grown under light and normal irrigations*

Kind of irrigation	Mean seedling stand of—	
	Pima	Acala
Light	4.6880±0.0213	3.3073±0.0213
Normal	3.9225± .0318	3.0804± .0278
Difference	+.7655± .0382	+.2269± .0350
$\frac{\text{Difference}}{E_{\text{diff}}}$	20.02	6.48

For both types of cotton, somewhat higher stands were secured with light irrigation at planting time. The difference is larger, and statistically far more significant, in the case of Pima Egyptian, where the difference is over 20 times as large as its probable error, than in the case of Acala upland, where the difference is about 6.5 times as large as its probable error.

The double χ^2 test (Table 11) shows in each case a probability of less than one in a million of the difference having arisen from random sampling. The χ^2 value indicates a greater significance in the case of the Pima Egyptian cotton. No final conclusion concerning the methods of planting to be recommended for agricultural practice should be attached to the results of this one experiment. The results are included here merely to indicate a line of investigation that is in progress and to show the applicability of criteria here proposed to a comparison of methods of planting as well as to a comparison of different agricultural varieties.

TABLE 11.—*Values of χ^2 and P for cotton grown under light and normal irrigations*

Variety and irrigation	χ^2	*P*
Pima (light and normal)	257.2815	0.000000
Acala (light and normal)	139.2729	.000000

DISCUSSION

The primary purpose of this paper has been to indicate methods of comparing the seedlings stands produced by two different varieties of cotton, or by the same variety as grown under two different cultural conditions.

Admitting freely that there are many possible methods of comparing the frequency distributions, the one recommended here for

the distributions as a whole is that of the double χ^2 test proposed by Pearson (*25*). This has the advantage of taking into account all the classes in the frequency distributions under consideration. The results secured in the present practical application of this method seem to justify fully the adoption of the procedure in cases where stringent comparisons are necessary.

The results of the double χ^2 test are supplemented by a comparison of the ratios of the differences between the mean number of seedlings per hill to their probable errors.

In the present investigation these ratios are generally so high and the values of P calculated from χ^2 are generally so small that it is impossible to bring them to a comparable quantitative scale without extending mathematical tables now available. Both of these criteria evidence highly significant differences in the capacities of varieties of cotton for the production of seedling stand.

Incidentally, comparisons are drawn between the seedling stands established by a number of different varieties of cotton. In order to determine finally whether all the differences found in these investigations are characteristic of the varieties, other experiments made with seed produced under the same environmental conditions are necessary. This is true because of the fact that the seeds here employed were drawn from different sources. This was both necessary and desirable from the standpoint of the main purposes of the experiments that yielded the data here considered. It is quite conceivable that some of the differences between varieties here found might disappear if the experiments were made with seed of the several varieties grown under exactly comparable conditions. In other words, it is possible that the differences observed may have been due to the peculiarities of individual samples of seed and that they were not typical of the varieties to which these seeds belong. The present results must therefore be considered as primarily a contribution to methods of investigation.

It seems clear, however, that under the conditions of these experiments, Pima Egyptian cotton produces a significantly better stand than upland varieties. This is apparent from the general consistency of the results for a large number of trials of Pima Egyptian cotton grown in comparison with various upland varieties.

Furthermore, under these conditions, sea-island cotton shows a behavior similar to that of the Egyptian type.

The superiority found in the smooth-seeded Pima variety as compared with the normal (parental) Pima type should be verified by further experimental comparisons before significance is attached to the result. This is true for the reasons indicated above—namely, that the differences found may have been due to slight differences in the particular samples of seeds employed rather than to a differentiation of the varieties.

Because of the limited number of comparisons between the upland varieties, final conclusions concerning the superiority of any individual variety with respect to its capacity for the establishment of seedling stand must await the results of other experiments.

The method of comparison suggested is obviously quite as applicable to the problem of determining the relative merits of different methods of planting, or irrigation, as it is to the problem of determining differences between varieties. The two illustrations given are in-

cluded merely for the sake of indicating methods of procedure in attacking problems of this kind.

C. J. King, superintendent of the United States Field Station at Sacaton, Ariz., to whom the writers are indebted for the facilities for these experiments, has pointed out that there have been a number of experiments at the station that indicate that the method of planting may influence the stand of seedlings. While for convenience the two methods employed by the writers have been designated as "normal" and "light" irrigation, the method of planting has other differences. The "light" irrigation plots were planted by the "ridge" method, while the other cultures were grown by the conventional flat or border planting system, with the flooding method of irrigation at planting time. King further notes that the "ridge" method of planting has proved to be advantageous under certain conditions and is practiced in Arizona in many areas where the soil is heavy, has a tendency to crust on wetting, or is slow in warming up in the spring.

It is difficult to determine, without further investigation, what are the precise factors that contribute to the differences observed in these experiments.

SUMMARY

The primary purpose of this paper is to give and to illustrate the applicability of criteria for the determination of differences in the seedling stands produced by different varieties of cotton when grown under the same conditions and by the same variety of cotton when grown under different field conditions.

Pearson's double χ^2 criterion, which compares all the classes involved in the two frequency distributions, is shown to be a suitable test of differentiation in such cases.

Incidentally, comparisons between the seedling stands produced by a number of varieties, as grown under irrigation in southern Arizona, are made. Pima Egyptian cotton showed a marked superiority over the upland varieties with which it was compared in its ability to establish seedling stands under the rather saline soil conditions of the Gila River Valley of southern Arizona. Sea-island cotton seemed to show a behavior similar to Pima Egyptian in this respect.

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SMUTS AND RUSTS PRODUCED IN CEREALS BY HYPODERMIC INJECTION OF INOCULUM[1]

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INTRODUCTION

As early as 1896 Hitchcock and Norton (*5*)[2] used a pointed glass tube to inject liquid cultures of *Ustilago zeae* into different parts of the maize plant. Since that time the method of inoculating maize by injection has been used frequently. Also, Faris and Reed (*2*) used this method in producing the loose kernel smut (*Sphacelotheca cruenta*) of sorghum in different parts of the plant, even though the only previously known means of infection by this organism was seedling penetration.

Because of the readiness with which certain strains of maize plants become smutted when a spore suspension of *Ustilago zeae* is hypodermically injected into or near the young terminal growing region of the plant (*4*), it seemed probable that slight variations in this method of inoculation might result in the production of smuts and rusts in small grains also.

INOCULATION WITH LOOSE SMUT OF BARLEY

Until 1924, it was thought that infection of barley by loose smut (*Ustilago nuda*) occurred only through the flower. In that year Tisdale and Tapke (*6*) obtained high percentages of loose smut by seed inoculation.

In the spring of 1927, plants of Tennessee Winter, Alaska, Alpha, Hannchen, and Nakano Wase barley were inoculated with spore suspensions of *Ustilago nuda*, when the plants were in the tillering stage. The spores had been soaked in distilled water for about 14 hours in order to hasten their germination and the growth of the germ tubes. The suspension was injected through the folded leaves and into or near the apical region of growth of the tillers. This region was very close to the ground.

Under artificial inoculation in the greenhouse, Tennessee Winter barley is highly susceptible to loose smut. The varieties Alaska and Alpha are susceptible to many collections of this organism. Hannchen is able to resist infection by spores of certain collections of loose smut, while it is extremely susceptible to others. Nakano Wase is very resistant.

[1] Received for publication Jan. 16, 1929; issued May, 1929.
[2] Reference is made by number (italic) to "Literature cited," p. 627.

After inoculation by the injection method, the expanding leaves were slightly shiny and yellowish green. This is characteristic of the leaves of young infected plants grown from hulled seeds inoculated with loose-smut spores. Many of the leaves showed whitish areas indicating a growth of mycelia in the leaf tissue, though no smut ever developed in these leaves.

When mature, 9 of the 27 inoculated plants of Tennessee Winter were smutted, while 45 noninoculated plants remained smut free. In experiments involving seed inoculated before sowing, diseased plants of this variety produced a high percentage of smutted tillers. However, when the inoculum was hypodermically injected some of the culms of smutted plants were healthy, indicating either the heading of new tillers which developed after inoculation or the escape of the inoculated tillers from smut.

Of 41 inoculated plants of the Alaska variety, 4 became smutted, while 46 noninoculated ones remained smut free. Less than 2 per cent of the inoculated plants of the Alpha variety developed smut, and no smut occurred in the control. Hannchen showed only one smutted inoculated plant, and a single smutted plant appeared also in the control. Nakano Wase produced no smutted plants in either the inoculated or the noninoculated rows. It is possible that a modification of this injection method or the development of a finer technic in inoculating the plants would result in increasing the percentage of smutted plants.

It is not unreasonable to suppose that the young tissues in the apical growing points of cereals are susceptible to smut when inoculated with a spore suspension by means of a hypodermic needle, for this tissue is very tender and only a little farther advanced in growth at this stage than in the germination stage in which the normal infection occurs.

Oats, normally infected by inoculating the seed or the flower, have been smutted by an artificial method by Gage (*3*). In a study of the growth relations between host and parasite he inoculated oat plants about 6 inches high by applying the oat-smut (*Ustilago* sp.) spores in the region of the first node. He did not state whether the inoculum was injected into the node or the spores were applied externally, but probably referred to the former. When mature, six of the nine plants thus inoculated were smutted, while the controls were smut free.

Recent experiments to determine the pathogenicity of plus and minus strains of smuts of small grains indicate the need of different methods of inoculation. Dickinson (*1*), using monosporic cultures of the covered smuts of oats and barley, inoculated seedlings having coleoptiles 5–10 mm. long by rubbing the inoculum on the coleoptile. He determined infection by the penetration of the smut organism, though penetration or infection by a smut organism does not mean the ultimate production of smut. Similar results were obtained also by wound infection and by injection with a hypodermic needle. These methods, however, he discarded in favor of the simpler rubbing method. By means of the injection method, though, it might be possible to study the smut reaction in older plants, especially those of resistant varieties, as well as to use it for pathogenicity studies.

INOCULATION WITH CORN RUST

In order to determine whether the injection method of inoculation might be convenient and useful in experiments on corn rust (*Puccinia sorghi*), several dent-corn plants growing in the greenhouse were injected near the top of the stalk with a suspension of urediniospores in distilled water. As the plants had not yet tasseled, the spores came in contact with the young, upper leaves, stalk, and tassel. Later, when the leaves unfolded, they were found severely rusted. The infection resulting from this inoculation was much more severe than that developed on the same lines of corn from natural infection in the same greenhouse.

INOCULATION WITH WHEAT RUST

Young wheat plants of the Purplestraw, Hard Federation, Harvest Queen, and Turkey varieties were inoculated by injecting a distilled-water suspension of urediniospores of leaf rust (*Puccinia triticina*) with a very fine hypodermic needle into the tillers near the crown. Although some of the suspension leaks from the puncture and runs down the side of the culm and some is forced out of the top, there is no general distribution of rust spores. Other plants were inoculated by spraying a part of the same suspension of rust spores on the leaves with an atomizer. The leaves were not rubbed before spraying. Some of the plants inoculated by each method were placed immediately in a small glass-inclosed chamber in which the humidity was kept high by frequently watering the sides and the floor. The rest of the plants were left under ordinary greenhouse conditions. About seven days later, flecking was noted on the leaves of the hypodermically inoculated plants; and about a week later many of the leaves were abundantly rusted. The plants maintained under ordinary greenhouse conditions were as abundantly rusted, however, as those kept in the humidity chamber. No pustules were found on any of the sprayed plants either within the incubation chambers or on the greenhouse bench.

The foregoing experiment was repeated, using the varieties Hard Federation, Harvest Queen, and Turkey. In addition, a series of the same varieties was inoculated with urediniospores of stem rust of wheat (*Puccinia graminis tritici*). It was found that all plants hypodermically inoculated with spore suspensions of either *P. triticina* or *P. graminis tritici* were abundantly rusted. The plants sprayed with leaf-rust spores all remained rust free, while those sprayed with stem rust developed but a few pustules.

Later, older plants of the Harvest Queen and Turkey varieties were inoculated with stem rust by the injection and the spray methods. The leaves of the plants to be sprayed were wet with distilled water and drawn between the thumb and forefinger in order that more of the spore suspension might remain on the leaves. Abundant infection was obtained again in the hypodermically injected plants, though no pustules developed on the sprayed plants. The severity of the infection on one of the injected Harvest Queen plants is shown in Figure 1.

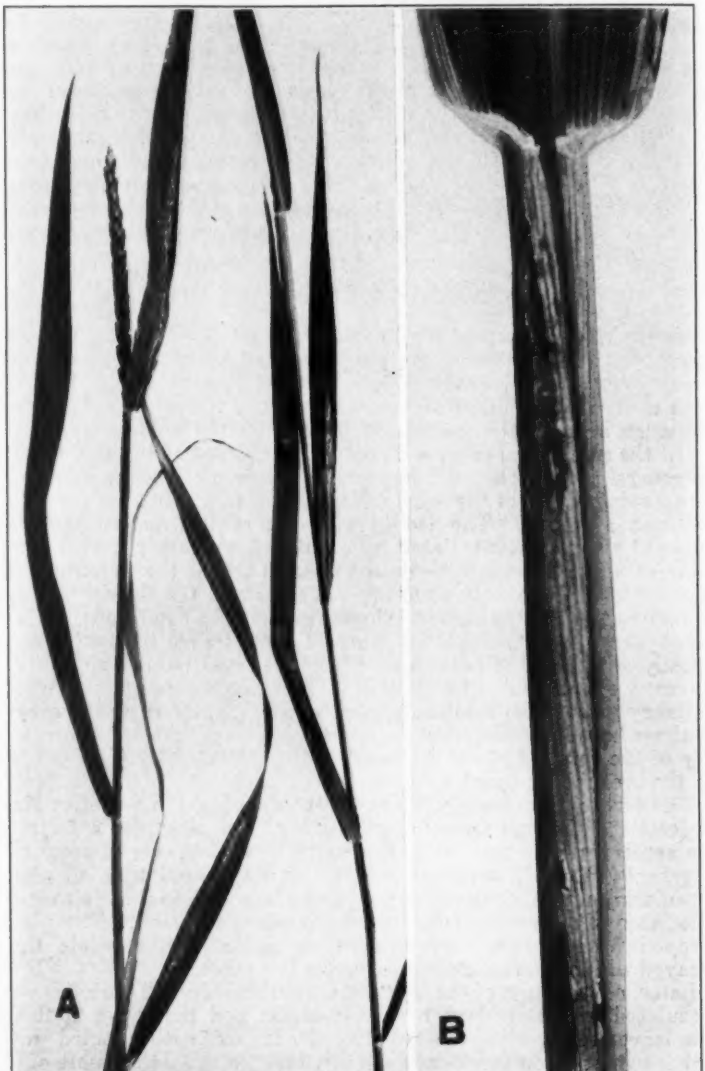

FIGURE 1.—A, Harvest Queen wheat in heading stage, showing stem-rust infection on leaf and leaf sheath induced by hypodermic injection of urediniospores into the region of the growing point; B, enlarged view of infected leaf sheath from a Harvest Queen wheat plant, showing severity and type of infection

ADVANTAGES OF INJECTION METHOD OF INOCULATION

In addition to the advantage of greater certainty of successful infection under ordinary conditions, there are other advantages in the use of the injection method for different rusts. Waters (7), in a recent paper on rusts, mentions the difficulty he encountered in spraying rust-infected plants to control mildew, as the ordinary disinfectants killed the rust inoculum as well as the mildew. No such difficulty would be encountered if the hypodermic-injection method of rust inoculation was used, as the effective inoculum is in between the leaves. In pathogenicity experiments requiring the testing of each of several rust collections, this method would help in keeping the collections separate. It may prove valuable also in facilitating infection of the barberry and other alternate hosts involving injection of suspensions of sporidia.

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APHODIUS GRANARIUS (COLEOPTERA), AN INTERMEDIATE HOST FOR HYMENOLEPIS CARIOCA (CESTODA)[1]

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INTRODUCTION

During the collection of material for experimental work on poultry cestodes, specimens of a dung beetle, *Aphodius granarius* (identified by E. A. Chapin, Bureau of Entomology, United States Department of Agriculture), were found frequently in chicken runs at the United States Animal Husbandry Farm, Beltsville, Md. By feeding gravid segments of *Hymenolepis carioca* to beetles and subsequently feeding the infected beetles to chickens, it was found that *A. granarius* serves as an intermediate host for *H. carioca*. Cysticercoids of *H. carioca*, which have not been described previously, were found occasionally in natural infestations in the beetles, as well as after artificial feedings. A preliminary note on this was published by Jones;[2] this paper supplies the detailed evidence. Previous work on the life history of *H. carioca* by Guberlet[3] incriminated the stable fly, *Stomoxys calcitrans*, as an intermediate host on the basis of feeding experiments; cysticercoids were not found in nor described from the fly.

Cysticercoids were present in the body cavity, and also in the tissues of *Aphodius granarius*. The period of development probably varies with temperature conditions, as other observers have noted in the case of tapeworms, but no temperature records were noted by the writer; 18 days was the minimum time, after the beetles were given cestode material, in which cysts were observed on dissection and later proved to be infective. Mature cysticercoids (fig. 1) are 200μ to 225μ in length and 180μ to 185μ in width. The scolex, unarmed and with saclike rostellum, measures 120μ to 130μ in width. The appendage or tail is prominent, with embryonic hooks at the tip or scattered along one side; the hooks are usually lost by the time the cysticercoid reaches maturity, as is true of other hymenolepid larvae described from various crustaceans. The tail may be several times the length of the cyst proper, or contracted and comparatively short, as in Figure 1.

PROTOCOLS

Chick 7583, hatched April 10, was fed on May 18 1 beetle to which segments of *Hymenolepis carioca* had been fed 21 and 23 days previously, and with cysticercoids which showed scolices with prominent suckers, but indefinite rostellum, present in the beetle on dissection. A few scolices evaginated before cysts were fed. Post-mortem examination of this chick on June 11, 24 days later, showed no helminths.

Chick 7565, hatched April 10, was fed beetles as follows: May 11, 1 beetle fed segments 16 days previously and with cysticercoids

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[2] JONES, M. F. PRELIMINARY NOTE ON THE LIFE HISTORY OF HYMENOLEPIS CARIOCA. Science (n. s.) 68: 512-513. 1928.

[3] GUBERLET, J. E. ON THE LIFE HISTORY OF THE CHICKEN CESTODE, HYMENOLEPIS CARIOCA (MAGALHAES) Jour. Parasitol. 6: [35]-38, illus. 1919.

present on dissection; May 14, 1 beetle fed segments 19 days previously and with cysticercoids present on dissection; May 22, 2 beetles fed

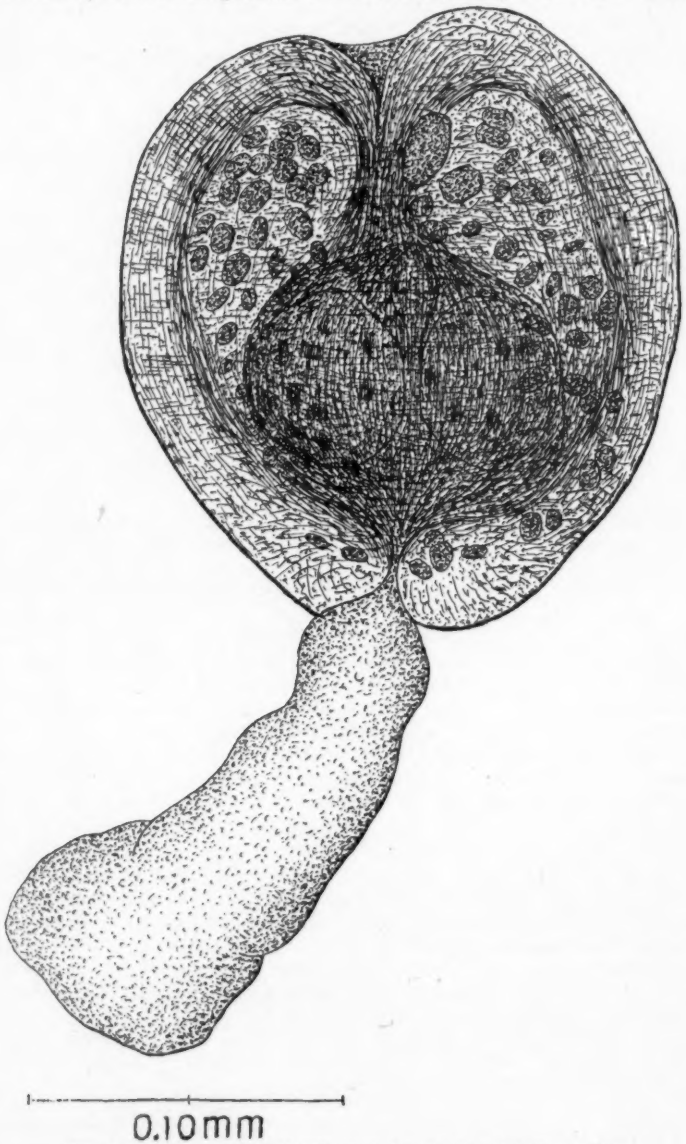

FIGURE 1.—Mature cysticercoid of *Hymenolepis carioca* from *Aphodius granarius*

segments 27 days previously and not examined. On post-mortem examination of this chick on June 13, 22 to 33 days after the feedings,

2 specimens of *Hymenolepis carioca* were present and seven gravid segments were loose in the intestine.

Chick 7759, hatched April 10, was fed beetles as follows: May 24, 1 beetle fed segments 30 days previously and not examined; 2 beetles fed segments 18 days previously and not examined (remainder of this lot of beetles examined and found to be negative); 1 beetle fed segments 16 days previously and not examined (remainder of this lot of beetles examined and found to be negative); 1 beetle fed segments 14 days previously and not examined; May 28, 2 beetles fed segments 17 days previously and not examined; June 1, 1 beetle fed segments 21 days previously and not examined. On post-mortem examination June 22, 21 to 29 days after feedings, 6 specimens of *Hymenolepis carioca* were found, 4 mature and 2 immature. No other helminths were present.

Chick 7581, hatched May 24, was fed 3 beetles as follows: May 28, 2 beetles fed segments 12 and 18 days previously, 1 dissected and with cysticercoids present, and 1 not examined; May 29, 1 beetle fed segments 12 and 18 days previously and not examined. On post-mortem examination June 20, 22 to 23 days later, 7 specimens of *Hymenolepis carioca* were present, all mature but with few gravid segments.

Chick 7584, hatched May 24, was fed 5 beetles as follows: May 28, 1 beetle fed segments 12 and 18 days previously and with cysticercoids present on dissection: May 29, 2 beetles fed segments 12 and 18 days previously, 1 with cysticercoids present on dissection, and 1 not examined; June 6, 2 beetles fed segments 21 and 27 days previously, 1 with cysticercoids present on dissection, and 1 not examined. Fecal examination of the chick on June 18, or three weeks from the date of the first feeding, showed the presence of gravid segments. Gravid segments were passed in numbers from that time until August 2, when the bird died. Post-mortem examination on that date showed 50 to 60 specimens of *Hymenolepis carioca*. The bird was thin, there were reddened areas along the intestinal wall, the vent was practically stopped, and the kidneys were greatly enlarged. One immature specimen of *Ascaridia lineata* was present. No cestodes other than *H. carioca* were found. Chicks 7581 and 7584 were 4 days old at the time of the first feedings of beetles, and mature worms were present when the birds were 25 days old. In other words, birds slightly more than 3 weeks old may harbor mature *H. carioca*.

To summarize these results it may be said that 5 birds were fed beetles; 4 became infected with *Hymenolepis carioca* and 1 remained negative. In all, 21 beetles were fed to the 5 birds; cysticercoids were demonstrated in 7 beetles and the other 14 were fed without dissection.

The birds of lot 1, the control chicks, hatched April 10, were examined as follows: 1 was examined June 14, and found negative for all helminths; 2 were examined June 15 and found negative for all helminths; 3 were examined June 26 and found negative for all helminths; 1 was examined August 8 and found negative for all helminths. Those in lot 2, hatched May 24, were examined as follows: 1 died June 15 and was found negative for all helminths; 3 were examined June 20 and found negative for all helminths; 3 were examined June 28 and found negative for all helminths; 1 was examined July 31 and found negative for cestodes but had 1 immature specimen of *Ascaridia lineata* present.

Some of the check birds were used in other feeding experiments; others were fed no invertebrate material. They were kept with the experimental birds, and as the birds grew larger they were kept in cages with not more than four in a cage, some controls being in each cage.

As a whole, of the 15 control birds of the same age and kept under the same conditions but not fed beetles, 14 were negative for all helminths, and 1 was negative for all cestodes but harbored one specimen of *Ascaridia lineata*.

CONDITIONS UNDER WHICH CHICKS WERE HELD

The first lot of chicks, hatched April 10, was kept in brooder boxes and then in a clean pen in one of the regular brooder houses at the experiment farm at Beltsville, Md. From May 1 the birds were kept in a screened room, in cages made of ½-inch wire mesh arranged so that droppings fell through the bottom and were not accessible to the birds. Fitted over each cage and dropping pan was a cover made of 2-mm. mesh copper screening. A small door in this permitted care of the chickens without the necessity of removing the screen cover. No helminths were found in any of the control birds of this lot.

The second lot, hatched May 24, was kept in a brooder box covered with a screen such as is described above, and later was moved to a cage similarly covered. On one occasion, owing to neglect in connection with the entrance of an electric cord to the brooder box, a small opening was left and a number of flies, *Musca domestica* and *Fannia* sp., were found under the cover. The chicks were under 2 weeks of age at the time and it seems improbable that any flies were consumed. In any case, since all chicks of this lot were together at the time, controls were under exactly the same conditions as experimental birds.

The food given to all birds, exclusive of the infective material fed individually to experimental birds, included neither fresh animal nor fresh vegetable material. With the exception of chick 7584, already discussed, and control chick 1100, which was given coccidia at one time, the birds remained in good condition throughout the experiment.

DISCUSSION

It seems evident that some poultry cestodes may have normally more than one intermediate host, possibly varying with different localities and with different seasons. Much remains to be determined regarding life cycles before control measures can be outlined in an adequate manner. At present, prophylaxis must be based on known facts, and to a limited extent on probabilities. Concerning the control of *Hymenolepis carioca*, the points to be emphasized in general sanitation are prompt and efficient disposal of droppings, so far as possible, and the keeping of yards free of unnecessary boards and miscellaneous objects that serve as temporary hiding places for beetles. If screening is resorted to, it is important that the mesh be fine enough to exclude small beetles as well as larger insects. Specimens of *Aphodius granarius* vary from 4 to 6 mm. in length and from approximately 1.5 to 3 mm. in width. Smaller specimens have been observed to pass through a 2-mm. opening easily, so that, according to present knowledge, only screening with a mesh of about 1.5 mm. as a maximum size prevents infection by the exclusion of the intermediate hosts already known.

THE EFFECT OF THE DIGESTIVE PROCESSES OF ANIMALS ON THE VIABILITY OF CORN AND SORGHUM SMUT SPORES[1]

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INTRODUCTION

Feeding cornstalk fodder and sorghum grain to livestock is a common farm practice, and since these feeds are often smutted, the question of the possible influence of such a practice upon the dissemination and perpetuation of the smuts, as well as the poisonous effect of the smuts when eaten by animals, becomes important from both theoretical and practical points of view. There is considerable literature on these questions regarding corn smut, but there is still lack of definite information. As far as the writers have been able to learn there is no information of this sort available regarding sorghum smut.

It seemed desirable, therefore, to test the viability of the chlamydospores of corn and sorghum smut after they had passed through the digestive tract of animals, and to make close observations as to the poisonous effect of these spores when eaten. It was considered that any data obtained in a study of this kind would be of value in helping to gain a better knowledge of the method of dissemination and perpetuation of these spores. It was also thought that any information on the relative danger of feeding smutted cornstalk fodder and smutted sorgum grain to livestock would be of considerable value.

REVIEW OF LITERATURE

It is a well-known fact that the seeds of some noxious weeds pass through the digestive tract of animals uninjured, and their germination may even be promoted by the process. According to Brefeld (*2, p. 29*),[2] smut spores are favorably influenced and their germination promoted by passing through the alimentary canal. Tests made by other German investigators do not substantiate this report with reference to bunt spores, *Tilletia foetens* (B. and Q.) Tre. Tubeuf (*12*) found that spores of stinking smut of wheat were for the most part destroyed in passing through the digestive tract. Honcamp, Zimmerman, and Schneider (*6*) made a study of the possibility of wheat infection by bunt spores evacuated by animals. They found that spores passing through the digestive tract had, in great part, lost their germinating power, and that the loss was less pronounced in case of swine than with other animals.

Most of the experiments in regard to the feeding of corn smut have been in relation to the study of the so-called cornstalk disease of

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[2] Reference is made by number (italic) to "Literature cited," p. 645.

cattle, but a few observations regarding the germinating power of the chlamydospores after passing through the digestive tract were made. In experiments conducted by Morini (*8a*), and by Arthur and Stuart (*1*), corn smut was fed to cows and a microscopic examination made of the spores evacuated by the animals. According to the former author the evacuated spores were viable, but according to the latter they were not. The investigators used only one animal in their experiment. As far as the writers have been able to determine, there are no other investigations on this phase of the problem.

METHODS AND MATERIALS

The studies herein reported consisted of feeding corn and sorghum smut to cattle and horses followed by testing the spores in the excreta for germination. The viability of the spores from the same lot was determined both before and after feeding.

Most of the corn smut used in the experiments of 1925–26 was collected in the corn-smut nursery of the Department of Botany and Plant Pathology of the Kansas State Agricultural College, and that used in 1926 and 1927 from several cornfields near Manhattan, Kans., and from smutted plants in the corn-smut nursery. The covered kernel smut of sorghum used during both seasons was obtained from a large number of sorghum varieties grown by the Department of Botany and Plant Pathology. As soon as the corn-smut boils and the smutted sorghum heads were collected they were spread on a floor to dry for several weeks, and were afterwards stored in barrels and sacks. The smut spores were separated from the extraneous material by crushing and rubbing the smut boils or smutted sorghum heads over a heavy screen, followed by rescreening through a finer mesh. Each lot of smut was divided into several 4-pound portions and, after representative samples were removed for checks and germination tests, was stored in closed paper bags until it was fed.

A considerable amount of sorghum smut was also fed in the form of entire smutted heads, a few of the smutted kernels being removed from each head for germination tests. In these experiments a total of 73.5 pounds of corn smut, 35.76 pounds of screened sorghum smut, and 1,793 smutted heads of sorghum, was used.

The collection of the samples of feces was accomplished by three methods: (1) The feces were placed in a clean, closed container after being dropped in the gutter; (2) the surface portion of the dung voided by the animal was removed and the sample taken near the center; (3) the excreta was removed from the rectum before natural evacuation. The amount of feces used in the final sample was governed by experience and the quantity of smut fed to the animal.

The final sample was made into an infusion by mixing it with distilled water. The mixture was then strained through a fine-mesh cloth, which retained the coarse material in the feces and allowed the spores to pass. The spore suspension was centrifuged for several minutes, the liquid decanted, and the spores at the bottom removed for germination tests. The spores were then placed in a 5 per cent solution of copper sulphate and shaken vigorously for one to two minutes in order to kill the spores of other fungi, since it had previously been found that this strength of copper sulphate did not injure these smut spores even when they remained in the solution for five

minutes. The control samples were treated in the same manner as the spores recovered from the feces. Evacuated spores were also tested for their germinating power without being treated with the solution of copper sulphate. The centrifuging method was again used to separate the spores from the copper sulphate solution, after which they were washed twice in distilled water before being tested for germination.

Sorghum-smut spores germinate readily in water, making it unnecessary to use a special medium. Germination of corn-smut spores, however, was found to be very irregular in water so that a special nutrient solution was used for all germination tests of these spores. This solution, a modified Cohn's solution with a pH of 4.8, was made by dissolving in 169.54 cubic centimeters of distilled water 14.0 grams of cane sugar, 1.0 gram of ammonium tartrate, and 0.5 gram of each of potassium sulphate, magnesium sulphate, and calcium phosphate ($Ca(H_2PO_4)_2$). The solution was sterilized for 20 to 30 minutes under 15 pounds pressure in an autoclave, stored under aseptic conditions and removed in small amounts as needed. The chlamydospores were tested for their germinability in Petri dishes, after the nutrient solution (in the case of corn-smut spores) or water (in case of the sorghum-smut spores) had been added. These cultures were incubated in moist chambers at a temperature of 28° C. to 30° C. The controls were treated in the same manner in all respects as those recovered from the feces. The chlamydospores of the controls of sorghum smut usually germinated within 12 hours and those of corn smut within 24 hours. Accordingly observations on the germinability of the spores of sorghum smut were made between 12 and 24 hours, and those on corn smut between 24 and 48 hours of incubation.

FEEDING CORN SMUT TO CATTLE

Corn smut collected during the summer of 1925 was fed to cattle during February, 1926, while that collected during 1926 was fed during December of the same year.

The first experiment of feeding corn smut to cattle was conducted in 1926 during a period of four days, from the 18th to the 21st of February, inclusive. The smut was fed to four different animals—No. 1 a heifer, No. 2 a dry cow, and Nos. 3 and 4 cows giving milk. In the first feed they all received one-half pound of smut with their grain ration. After the first feed the proportion of smut to grain or silage given to each animal was varied. The heifer, No. 1, received one-half pound of corn smut every meal, throughout the experiment. The amount of smut fed No. 2 was increased with each feeding, until she was fed 1 pound of smut with a pound of grain, twice a day. It was the intention to increase the proportion of smut to grain still more, but she refused to eat any more than this amount with her grain ration. In the case of cow No. 3, the 0.8 pound of smut fed the first day was decreased with each feeding until she received only 0.2 pound of smut per day. The amount of smut fed to cow No. 4 was decreased from 0.5 to 0.3 pound the second day. A daily record of corn smut fed to cattle and the germination percentages of evacuated spores are shown in Table 1.

The second experiment of feeding corn smut to cattle was conducted in 1926 during a period of five days, from the 8th to the 12th of

December, inclusive. Four animals (Nos. 7 to 10), ranging in age from 2 to 5½ years, were used. They were fed all the corn smut they could be induced to eat during the experiment. The amount of smut consumed varied from 0.4 to 1.75 pounds daily. The data obtained are shown in Table 1.

TABLE 1.—*Corn smut consumption records of cattle and the germination percentage of evacuated spores*

EXPERIMENTS WITH SPORES COLLECTED IN 1925

Animal No.	Feeding of—									
	Feb. 18		Feb. 19		Feb. 20		Feb. 21			
	Smut consumed	Germination of evacuated spores [a]	Smut consumed	Germination of evacuated spores	Smut consumed	Germination of evacuated spores	Smut consumed	Germination of evacuated spores	Smut consumed	Germination of evacuated spores
	Pounds	*Per cent*	*Pounds*	*Per cent*	*Pounds*	*Per cent*	*Pounds*	*Per cent*	*Pounds*	*Per cent*
1	1.0	0	1.0	0	1.0	0	1.0	0	------	------
2	1.1	0	1.8	0	2.0	0	2.0	0	------	------
3	.8	2.5	.5	0	.2	0	.2	0	------	------
4	------	------	------	------	.5	0	.3	0	------	------

EXPERIMENTS WITH SPORES COLLECTED IN 1926

	Dec. 8		Dec. 9		Dec. 10		Dec. 11		Dec. 12	
7	1.0	0	1.5	0	1.5	0	1.75	0	1.0	0
8	.4	0	.8	0	.8	0	.8	0	.4	0
9	.4	0	.8	0	.8	0	.8	0	.4	0
10	.4	0	.8	0	.8	0	1.0	0	.5	0

[a] Average percentage of germination of spores detected in all samples collected the same day. The average percentage of germination of 3 controls for 1925 was 75, and for 5 controls for 1926, 80.

The data in Table 1 show that no germination of corn-smut spores was observed in any of the samples taken from the excrements, except in the case of one sample from cow No. 3, where about 2.5 per cent of germination was detected. The average germination of all the controls in the first and second experiments was 75 and 80 per cent, respectively. These results show that the spores of *Ustilago zeae*, almost without exception, lose their viability after passing through the digestive tract of cattle and indicates that feeding corn smut to these animals can not be a significant factor in spreading this disease. Some viable spores may, however, get into the stable manure by handling smutted feed in the barn or feed lot, and it is very liklely that such spores remain viable if the manure is taken directly to the field.

FEEDING CORN SMUT TO HORSES

Experiments in the feeding of corn smut to horses were also conducted during two years, in which different animals were fed with smut produced during two different seasons. The smut fed was mixed with bran and ground corn. In the 1926 experiment the amount of smut fed to the animals was gradually increased, while in 1927 the horses used were from the beginning given all the smut they could be induced to eat.

The first feeding experiment was conducted in April, 1926. Horse No. 1 was fed corn smut over a period of 11 days, and Nos. 2 and 3 were fed smut for 2 days. The two latter received 2 ounces a feed or 4 ounces a day. It was thought that the amount of smut fed might have some influence on the number of viable spores passing through the digestive tract. Accordingly only 1 ounce of smut mixed with bran mash was given horse No. 1 the first feed, but increased 2 ounces a day until the ninth day, when the animal consumed 18 ounces, or about 2 quarts of smut, the same rate of consumption being continued for the next 2 days.

In the second experiment use was made of five horses—Nos. 4, 5, 6, 7, and 8. Horse No. 4 was fed corn smut continuously for 12 days, from January 27 to February 7, 1927. During the first week the animal consumed one-fourth pound of smut mixed with bran twice daily. From that time on the amount was gradually increased to one-half pound twice daily until the twelfth day, when the horse was killed. Horses Nos. 5 and 6 were first fed smut on February 15. Horse No. 5 consumed one-fourth pound and No. 6 three-fourths pound. They seemed to like the smut and were given all they would eat at each feeding. The fifth day each animal consumed 3 pounds. Horse No. 6 was killed February 21, and No. 5 was used for other experimental purposes after February 22. At this time two ponies, numbered 7 and 8, were secured and fed one-half pound of smut with bran night and morning. Animal No. 7 was fed for a week and killed February 28, while No. 8 was fed until March 5, having been fed for a period of 12 days. The amount of corn smut consumed by horses in the 1926 and 1927 experiments and the germination percentages of evacuated spores are shown in Table 2.

TABLE 2.—*Corn-smut consumption records of horses and the germination percentages of evacuated spores, 1926 and 1927*

Animal No.	Date of feeding	Pounds of smut consumed	Number of samples examined for spores	Number of samples in which germination was detected [a]	Viable spores detected [b]					
					Date	Per cent	Date	Per cent	Date	Per cent
	1926									
1	Apr. 7–Apr. 18	8.00	21	3	Apr. 14	0.1	Apr. 15	0.2	Apr. 17	0.1
2	Apr. 16–Apr. 18	.50	9	0						
3	do	.50	9	0						
	1927									
4	Jan. 27–Feb. 7	6.50	21	0						
5	Feb. 15–Feb. 22	8.77	15	3	Feb. 21	2.0	Feb. 22	1.0	Feb. 23	Trace.
6	Feb. 15–Feb. 21	9.25	14	1			do	Trace.		
7	Feb. 22–Feb. 28	3.75	10	0						
8	Feb. 22–Mar. 5	5.75	24	0						

[a] The average percentage of germination of the controls was 75 and 81 for 1926 and 1927, respectively.
[b] Average percentage of germination of spores detected in all samples collected the same day.

The data in Table 2 show that in the case of only three animals were any viable spores recovered from the feces. The percentage of germination, however, was small in these cases and ranged from a trace to 2 per cent. These viable spores came only from horses which were being fed from 1 to 3 pounds of smut daily. When it is con-

sidered that the germination of the controls ranged from 75 to 80 per cent, it is seen that the viability of the evacuated spores in these two cases is insignificant.

FEEDING SORGHUM SMUT TO CATTLE

The experiments of feeding covered kernel smut of sorghum to cattle were conducted during March and December, 1926. The smut fed during the first experiment was produced during 1925, while in the second experiment, smut of the 1926 crops was used. The animals fed were all of the Jersey breed. Since it was thought probable that feeding whole, smutted heads of sorghum instead of spores in powdered form might make a difference in the number of viable spores recovered, some of the animals were fed the latter, while others received whole smutted heads, or the same animals were fed both the powdered smut and whole smutted heads.

The first experiment lasted eight days. The feeding of four animals was started on the afternoon of March 9, 1926. The cows were fed screened sorghum-smut spores with the grain ration, twice daily. Besides the smut and grain the animals received alfalfa hay and corn silage. The last day two additional cows, Nos. 5 and 6, were fed whole smutted heads. The amount of smut powder fed to animals 1, 2, 3, and 4 was increased from 0.1 to 0.2 pound after the first feeding. Animals Nos. 5 and 6 each consumed 40 smutted heads in the morning and 100 smutted heads at night during the last day of the experiment. They did not eat all the alfalfa given them that night, but seemed to relish the smutted heads of sorghum.

The second experiment was conducted in December, 1926. The smut was fed to seven different animals—dairy cows and heifers. In the first part of this experiment four cows were fed all the powdered smut they would eat. In the second part an effort was made to induce the animals to eat whole, smutted heads of sorghum in addition to the powdered form, but only two would eat them. From previous feeding it was found that heifers, as a rule, would eat smutted heads of sorghum, so, accordingly, three young animals were selected and fed all the smutted heads they would eat. They were induced to eat more at each subsequent feeding, so that the last time they were fed they each consumed 75 smutted heads. A record of sorghum smut consumed by cattle and the germination percentage of evacuated spores is given in Tables 3 and 4.

TABLE 3.—*Sorghum smut consumption record of cattle and the germination percentages of evacuated spores in experiment with smut collected in 1925* [a]

Animal No.	Feeding of—							
	Mar. 9		Mar. 10		Mar. 11		Mar. 12	
	Smut spores consumed	Germination of evacuated spores [b]	Smut spores consumed	Germination of evacuated spores	Smut spores consumed	Germination of evacuated spores	Smut spores consumed	Germination of evacuated spores
	Pound	*Per cent*	*Pound*	*Per cent*	*Pound*	*Per cent*	*Pound*	*Per cent*
1	0.3	5.6	0.4	Trace.	0.4	Trace.	0.4	1.50
2	.3	Trace.	.4	Trace.	.4	0	.4	2.00
3	.3	Trace.	.4	Trace.	.4	0	.4	5.17
4	.3	Trace.	.4	Trace.	.4	0	.4	.20
5								
6								

[a] The average percentage of germination of 5 controls in the 1925 experiment was 95.
[b] The average percentage of germination of spores detected in all samples collected the same day.

TABLE 3.—*Sorghum smut consumption record of cattle and the germination percentages of evacuated spores in experiment with smut collected in 1925*—Continued

Animal No.	Feeding of—										
	Mar. 13			Mar. 14			Mar. 15			Mar. 16	
	Smut spores consumed	Smut heads consumed	Germination of evacuated spores	Smut spores consumed	Smut heads consumed	Germination of evacuated spores	Smut spores consumed	Smut heads consumed	Germination of evacuated spores	Smut heads consumed	Germination of evacuated spores
	Pound	*Number*	*Per cent*	*Pound*	*Number*	*Per cent*	*Pound*	*Number*	*Per cent*	*Number*	*Per cent*
1	0.4	.....	2.15	0.4	.....	0	0.4	.....	0	.....	.....
2	.4	.....	.33	.4	.....	0	.4	.....	0	.....	.....
3	.4	.....	0	.4	.....	0	.4	.....	0	.....	.....
4	.4	.....	0	.4	.....	0	.4	.....	0	.....	.....
5	.....	20	1.50	.....	20	1.75	.....	20	0	140	0
6	.....	20	6.87	.....	20	.65	.....	10	0	140	0

TABLE 4.—*Sorghum smut consumption record of cattle and germination percentages of evacuated spores in experiment with smut collected in 1926* [a]

Animal No.	Feeding of—										
	Dec. 25		Dec. 26		Dec. 27			Dec. 28			Dec. 29	
	Smut spores consumed	Germination of evacuated spores [b]	Smut spores consumed	Germination of evacuated spores	Smut spores consumed	Smutted heads consumed	Germination of evacuated spores	Smut spores consumed	Smutted heads consumed	Germination of evacuated spores	Smutted heads consumed	Germination of evacuated spores
	Lbs.	*Per cent*	*Lbs.*	*Per cent*	*Lbs.*	*No.*	*Per cent*	*Lbs.*	*No.*	*Per cent*	*No.*	*Per cent*
7	0.50	0.01	1.25	Trace.	0.75	60	3.30	0.24	22	Trace.	20	Trace.
8	.50	0	1.25	0.03	1.25	40	Trace.	.50	50	Trace.	20	0
9	.50	0	1.25	0	1.25	.....	0	.50	.....	0	.....	.....
10	.51	0	1.25	Trace.	1.12	.....	0	.50	.....	0	.....	.....
11	.....	.....	.....	.....	.....	15	0	.....	52	0	75	0
12	.....	.....	.....	.....	.....	10	0	.....	60	Trace.	75	0
13	.....	.....	.....	.....	.....	10	0	.....	60	0	75	0

[a] The average percentage of germination of 8 controls in the 1926 experiment was 95.8.
[b] The average percentage of germination of spores detected in all samples collected the same day.

Out of 70 samples of evacuated spores tested in the first experiment, 26 germinated to some extent. Of this number 17 showed only a trace (less than 1 per cent), while the other 9 samples showed from 1 to 13 per cent of germination. The average percentage of germination of the spores in five controls was 95. In the second experiment 71 different samples of evacuated spores were tested for germination, but only 23 showed any viability. Twenty-two of the 23, showed only a trace (less than 1 per cent), and the other sample showed 3.3 per cent germination. The average percentage of germination of the spores taken from the same material before it was fed was 95.8. These results show that the survival of the spores after passing through cows is very small, and in only a few cases is the percentage significant.

FEEDING SORGHUM SMUT TO HORSES

Kernel smut of sorghum was fed to four different horses, Nos. 1, 2, 3, and 4, during the last four days of December, 1926, and first day of January, 1927.

Screened smut and smutted heads were consumed by these animals during a period of four days. Besides this material, horses Nos. 1, 2, and 3 received sorghum and millet forage, while No. 4 received prairie hay and grain. The amount of smut consumed daily by the individual animals and the viability of the evacuated spores is shown in Table 5.

TABLE 5.—*Sorghum smut consumption records of horses and the germination of evacuated spores, 1926–27*

Animal No.	Feeding of—												
	Dec. 28, 1926		Dec. 29, 1926		Dec. 30, 1926			Dec. 31, 1926			Jan. 1, 1927	
	Smutted heads consumed	Germination of evacuated spores[a]	Smutted heads consumed	Germination of evacuated spores	Smut spores consumed	Smutted heads consumed	Germination of evacuated spores	Smut spores consumed	Smutted heads consumed	Germination of evacuated spores	Smutted heads consumed	Germination of evacuated spores
	No.	*Per cent*	*No.*	*Per cent*	*Lbs.*	*No.*	*Per cent*	*Lbs.*	*No.*	*Per cent*	*No.*	*Per cent*
1					1.24	40	Trace.	2.0	90	Trace.	70	Trace.
2					1.24	40	Trace.	2.0	90	0	70	0
3					1.24	40	Trace.	2.0	70	Trace.	50	Trace.
4	2	0	27	Trace.	1.12	15	0	2.0	90	0	55	0

[a] The average percentage of germination of 6 controls was 96 per cent.

The experiment on feeding covered kernel smut of sorghum to horses shows that practically all spores passing through the alimentary canal of the horse are killed, since in 47 samples only 13 showed any viability and then only a trace, while the average of germination of 6 controls was 96 per cent.

THE EFFECT OF THE DIGESTIVE JUICES ON THE VIABILITY OF SMUT SPORES

Determination of the hydrogen-ion concentration of the contents of the different parts of the digestive tract of horses was undertaken in order to find out whether the effect upon germination of smut spores might be due to the reaction to which they were subjected.

According to Smith (*11*) pure gastric juice of the horse has never been available for analysis, but lactic acid is said to be the chief digestive acid. Henry and Morrison (*5, p. 21–22*) in speaking of digestion in the stomach of a horse, state that the acid of the stomach is chiefly hydrochloric and varies in different animals from 0.2 to 0.5 per cent. They also mention that pepsin is most active in that portion of the stomach where hydrochloric acid is secreted.

Considerable work has been done on the effect of hydrogen-ion concentration on the growth of fungi. Currie (*3*) found that the critical acid concentration for *Aspergillus niger* ranges from pH 1.4 to 1.6. According to Meacham (*8*) the limiting pH value for *Lenzites sepiaria*, Fomes, and Coniophora is near 1.7.

Piemeisel (*9*) found that spores of *Ustilago zeae*, almost without exception, lost their viability after having been kept in a silo for a few weeks, and suggested that the destructive agents were probably the silage acids, especially acetic. The total acidity of corn silage, according to Esten and Mason (*4*) is about 1.0 to 1.5 per cent. The principal acids are said to be lactic, acetic, and proprionic. It should be kept in mind that the injurious effect of any acids probably depends to a large extent on their dissociation or the concentration of H ions in solution. Platz, Durrell, and Howe (*10*) found that carbonic acid when present in concentrations of pH 4.5 to 5.6, had a stimulating effect on chlamydospore germination of *U. zeae*. Leszczenko (*7*) germinated spores of *U. zeae* in aqueous solutions of acids with nutrients of 0.1 per cent peptone plus 0.1 per cent grape sugar at a temperature of 25° C. He found that corn smut spores do not germinate in acids of the following concentrations: N/80 HNO_3, N/60 H_2SO_4, N/45 HCl, and N/10 H_3PO_4.

In our experiments the contents of the stomach and duodenum, near the pylorus, were found to be acid; those of the small intestines, alkaline, and those of the other parts, either slightly acid or slightly alkaline, as shown in Table 6.

TABLE 6.—*Hydrogen-ion concentration of contents of the digestive tract of horses, eight hours after the death of the animal*

Date	Horse No.	Hydrogen-ion concentration, pH of the contents of the—					
		Stomach	Duodenum	Small intestines	Cecum	Colon	Rectum
Feb. 14	4	2.6	2.8	8.1	7.4	7.0	6.2
Feb. 21	6	2.7	3.0	8.1	6.5	7.2	7.3
Feb. 28	7	2.6	2.8	8.6	7.6	7.5	6.4
Mar. 7	8	2.6	2.8	8.4	7.2	7.2	6.2

Having found in the studies herein reported, that most of the smut spores fed are killed in the process of digestion, it was thought desirable to learn what part of the digestive tract had the most influence on their vitality. Accordingly, portions of the contents of the different parts of the digestive tract of five horses, fed with corn smut, were collected from seven to eight hours after the animal had been killed. The spores were removed from the extraneous material and tested for their germination. Spores taken from the contents of the small intestines, colon, and rectum did not show any germination, while some of the spores removed from the contents of the stomach near the cardia, and some taken from the contents of the cecum, were found to be viable. Horse No. 4 was fed 0.5 pound of powdered corn smut with ground corn one hour before he was killed. Upon examination the stomach was found to be almost full, and the food was arranged in layers. The top layer consisted of a mixture of corn smut and ground corn, apparently undigested. It was in this portion of the stomach contents that viable spores were found. Horse No. 6 was fed 1.5 pounds of corn smut mixed with grain about 12 hours before he was killed. Immediately after feeding, the animal was given about 5 gallons of water. It is very possible that some of the corn-smut spores were washed out of the stomach without being acted upon by the gastric juices. The data obtained in these experiments are shown in Table 7.

TABLE 7.—*Viability of corn-smut spores removed from the contents of the different parts of the digestive tract of horses, 1927*

Date	Horse No.	Relative amount of germination of spores from—				
		Stomach	Small intestines	Large intestines	Colon	Rectum
Feb. 7	4	Trace.	0	0	0	0
Feb. 14	9	Trace.	0	0	0	0
Feb. 21	6	Trace.	0	Trace.	0	0
Feb. 28	7	0	0	0	0	0
Mar. 7	8	0	0	0	0	0

In order to obtain further information in regard to the action of the digestive tract upon smut spores, an experiment was conducted in which sorghum smut was mixed with the contents taken from the stomach, cecum, and rectum, respectively. Owing to the fact that the animals had been fed corn smut before they were killed, sorghum-smut spores were used in this experiment, since they could easily be distinguished from the corn-smut spores already present in the contents of the digestive tract. From seven to eight hours elapsed after the death of the animal before the contents of the digestive tract were removed and the spores brought in contact with it. They were kept in these media during periods of from one-half to six hours at a temperature of 100° F. The spores were removed from the material, washed, strained out, and tested for germination in modified Cohn's solution at a temperature of 86° F., since these are known to be favorable environmental conditions for corn and sorghum smut spore germination. The results obtained are shown in Table 8.

TABLE 8.—*The relative germinating power of sorghum-smut spores after being in contact with the contents of the digestive tract of horses, incubated at 100° F., 1927*

Date	Horse No.	Number of hours of contact	Per cent of germination after being in contact with the contents of—			Date	Horse No.	Number of hours of contact	Per cent of germination after being in contact with the contents of—		
			Stomach	Cecum	Rectum				Stomach	Cecum	Rectum
Feb. 14	9	0.5	90–100	90–100	90–100	Feb. 28	7	0.5	90–100	90–100	90–100
		1.0	80–90	90–100	90–100			1.0	90–100	90–100	90–100
		1.5	40–50	90–100	90–100			1.5	80–90	90–100	90–100
		2.0	Trace.	90–100	90–100			2.0	40–50	90–100	90–100
		2.5	0	90–100	90–100			2.5	20–30	90–100	90–100
		3.0	0	90–100	90–100			3.0	5–10	90–100	90–100
		4.0	0	90–100	90–100			4.0	Trace.	90–100	90–100
		6.0	0	90–100	90–100			6.0	0	90–100	90–100
Feb. 21	6	.5	70–80	90–100	90–100	Mar. 7	8	.5	90–100	90–100	90–100
		1.0	50–60	90–100	90–100			1.0	80–90	90–100	90–100
		1.5	5–10	90–100	90–100			1.5	5–10	90–100	90–100
		2.0	2–5	90–100	90–100			2.0	Trace.	90–100	90–100
		2.5	Trace.	90–100	90–100			2.5	Trace.	90–100	90–100
		3.0	0	90–100	90–100			3.0	Trace.	90–100	90–100
		4.0	0	90–100	90–100			4.0	0	90–100	90–100
		6.0	0	90–100	90–100			6.0	0	90–100	90–100

Spores kept in the contents of the stomach from one and one-half to two hours showed a marked decrease in viability and in some cases only a trace of germination occurred. Spores in contact with the contents of the cecum or rectum for six hours did not show any loss in vitality under the conditions of the experiment.

THE PERMANENT NONVIABILITY OF EVACUATED CHLAMYDOSPORES OF CORN AND SORGHUM SMUT

Several tests and observations were made throughout these experiments to determine whether or not the chlamydospores of *Ustilago zeae* and *Sphacelotheca sorghi* after passing through the digestive tract became permanently nonviable, or whether germination was merely inhibited or retarded by the digestive process. In 1926 and 1927 feces of both horses and cattle containing evacuated chlamydospores were dried under natural conditions and the spores in this material were tested for viability after various times, but no germination was detected.

In another experiment the dried, evacuated spores were subjected to temperatures −15° C. to −20° C. for three hours. It was thought that low temperatures might activate the spores in manure. No germination was detected after 48 hours of incubation.

It seemed possible that seedling infection of sorghum might occur by contaminating the seed with sorghum-smut spores evacuated by animals. Accordingly the feces of cows to which sorghum smut had been fed were collected and dried at room temperatures and the spores in the feces were tested for their power to infect pink kafir after the following manner: The ground feces containing the spores were placed in a furrow 85 feet long in which the seed was planted. Under the conditions of the experiment no infection took place in this planting, while the control which was inoculated from the same sample that was fed showed 30 per cent of infected plants.

PALATABILITY OF SMUT AND ITS EFFECT ON ANIMALS

In feeding corn and sorghum smut to cattle and horses it was noticed that the appetite for this material varied considerably with the individual concerned. Some animals were indifferent about eating the smut, especially corn smut. Other individuals showed a decided preference for smut in the beginning, but after a day or two grew indifferent, although they continued to eat the mixture of smut and grain without apparent injury. As a rule the younger animals, or heifers, exhibited a greater appetite for smut than the older cows. It was observed that horses on a grain and prairie-hay ration consumed smut less freely than animals receiving only sorghum or millet forage.

The question that interests the farmer in connection with these studies is whether or not corn or sorghum smut when mixed with feed is poisonous or injurious to livestock. Maladies resembling the so-called cornstalk disease of cattle have not infrequently been charged to corn smut. In general, it has always been suspected as being harmful when fed to animals. In these studies 13 cows and 12 horses received smut. In this discussion these animals which were fed smut for only a short time, and hence consumed only small quantities of it, will not be considered, and only those individuals receiving the larger amounts of smut will be discussed. Six cows received 27.5 pounds of corn smut in 4 days, an average of 4.5 pounds per animal. This amounted to approximately 2 quarts of smut per day. Several cows consumed as much as 1.25 pounds of screened sorghum smut and others as much as 140 smutted heads a day. Eight horses consumed 43 pounds of corn smut, an average of 5.4

pounds an animal. These animals were fed smut for periods varying in length from 2 to 11 days. In another experiment ranging in length from 4 to 7 days, 4 horses received a total of 11.92 pounds of screened sorghum smut and 749 smutted heads, an average of 2.98 pounds of screened smut and 187 smutted heads an animal.

Under the conditions of the experiment the health of the animals to which corn and sorghum smut were fed did not seem to be impaired. Their appetites remained normal and there were no evidences of physiological disorders, with the exception that when smut was inhaled by horses it was irritating to the respiratory system, producing the same effect as feeding dusty hay. Dairy cows of different ages and in various stages of milk production and pregnancy were used in these experiments. The milk flow was in no way impaired nor were any other ill effects noted due to the consumption of smut.

SUMMARY

Corn and sorghum smut were fed to cattle and horses and the spores in their excreta were thereafter tested for germination. These tests were conducted during the winter and spring seasons of two years with dry smut collected from the crop of the preceding summer.

The experiments showed that the passage of chlamydospores of corn and sorghum smut fungi through the digestive tract of horses or cattle almost completely destroyed their viability. The survival of sorghum-smut spores is somewhat greater than the survival of corn-smut spores. Apparently the false membrane which covers the spore mass of covered kernel smut provides considerable protection. The germinating power of both corn and sorghum smut spores is not merely temporarily inhibited or retarded, but the spores become permanently nonviable. The number of spores which remains viable in the feces is so small that it can be considered a negligible factor in the perpetuation and spread of either of these diseases in the field.

The contents of different parts of the alimentary canal of several horses to which smut had been fed were examined in order to learn in what part of the digestive tract smut spores lose their germinating power. It was found that for the most part the spores lose their viability in passing through the stomach. This loss of viability is apparently due to the action of acids under favorable conditions in this organ. The acidity of the contents of the stomach of horses was found to be great enough to prevent the germination of nearly all spores when in contact with the contents for about two hours at body temperature.

There seemed to be no injurious effect from feeding either corn or sorghum smut to animals. Large amounts of both of these smuts were fed to cows and horses without apparent injury. Most of the animals to which smut was fed were carefully observed for several days after feeding and appeared normal in every respect. In fact, some individuals seemed to relish smut in their feed. At the beginning of the experiments some animals showed a decided preference for smut, but at the end of a few days they preferred other food, although they continued to eat smut without apparent injury.

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NATURAL CROSSING BETWEEN OAT PLANTS OF HYBRID ORIGIN [1]

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Evidence of the occurrence of natural crossing in oats grown on the agronomy farm near Morgantown, W. Va., was published [2] two years ago. In connection with an investigation of the inheritance of resistance to smut in the oat cross Gopher × Black Mesdag and the reciprocal, a considerable number of black-seeded plants were found among supposedly white-seeded families. These unexpected plants were thought to have been due to accidental mixtures or to natural crossing. In all, there were 15 black-seeded plants growing among forms supposed to be homozygous for white seed, and of the 15 plants 14 segregated for seed color in the next generation, showing conclusively their hybrid origin. One of the plants bred true for black seed, and therefore it probably was the only one of this group whose presence was due to an accidental mixture.

Assuming that the 14 black-seeded plants were produced by natural crossing, the cross-pollination which produced them must have occurred the year previous to that in which they were observed (1927). The history of these plants shows that in every case the strains in which they occurred were growing adjacent to black-seeded plants the previous year. Some of the natural hybrids occurred in strains heterozygous and some in strains homozygous for seed color in 1926.

TABLE 1.—*Brief history of certain white-seeded F_4 families of oats in which black-seeded plants were found*

Designation of F_4 family	Position in the nursery in F_3 in 1926	Number of plants and color of seed in 1927		Designation of F_4 family	Position in the nursery in F_3 in 1926	Number of plants and color of seed in 1927	
		White	Black			White	Black
16–15–7–5	[a] S S W	58	1	16–15–64–3	S S W	63	2
16–15–39–5	W S B	60	1	16–15–64–4	S S W	45	2
16–15–31–2	B W S	57	2	16–15–68–4	S W S	57	1
16–15–31–4	B W S	60	1	17–10–21–1	S S W	58	1
16–15–16–5	S W W	56	1	17–10–52–4	W S B	57	1
16–15–17–2	W S W	62	1	17–10–53–4	S W S	56	1

[a] B = black seed, W = white seed, and S = segregation for seed color. The middle letter describes the condition of the particular strain in which the black-seeded plant was found. The other two letters indicate the condition of the strains growing in adjacent rows.

In Table 1 is given a short history of the strains in which the black-seeded plants were found. For example, the F_3 family, 16–15–7, segregated for seed color in 1926. The family grown on one side of this strain segregated for seed color and the family on the other side had white seed. One of the white-seeded plants selected from this F_3

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[2] GARBER, R. J., and QUISENBERRY, K. S. NATURAL CROSSING IN OATS AT MORGANTOWN, WEST VIRGINIA. Jour. Amer. Soc. Agron. 19: 191–197. 1927.

family and grown in F_4 (16–15–7–5) produced 58 white-seeded and 1 black-seeded plant. Similarly, the history of the other strains in which black-seeded plants were found is shown.

It will be observed from the second and fourth columns of Table 1 that nine of the black-seeded plants came from families which segregated in F_3, and six from families which supposedly were homozygous for white seed. Each F_4 family, of course, consisted of the progeny of a single white-seeded F_3 plant. In view of the close proximity of black and white seeded plants to one another it is most probable that the black-seeded descendants of white-seeded parents may, with a single exception, be attributed to natural crossing.

There were numerous other white-seeded F_4 families grown in 1927 which had a comparable history to those reported above but which did not show any black-seeded plants. Of a total of 5,132 F_4 plants belonging to families supposedly homozygous for white seed, 14 plants had black seed and segregated for seed color in the next generation. The 5,132 plants came from sources with similar opportunities for natural crossing between black and white seeded forms. If it is assumed that there were as many reciprocal natural crosses as there were crosses observed among the white-seeded forms, the percentage of natural crossing which led to a genetic contamination among the F_3 families in 1926 was 0.54 per cent. Perhaps it should be pointed out that the oats grown in connection with the inheritance studies of resistance to smut carried on at West Virginia are not planted until about June 15, which fact may be of some significance in explaining the relative amount of natural crossing. Oats planted at this time bloom in August, when the weather conditions at Morgantown are generally more favorable for natural hybridization than in June. In the earlier investigation,[3] which had for its object the determination of the extent of natural crossing between varieties of *Avena sativa* planted at the usual time, only 1 natural hybrid was found among a total of 7,742 plants.

In addition to the natural hybrids observed among the F_4 families, one was found in a plot of the Gopher parent. This one is not included in calculating the percentage of natural crossing because the individual plants of the plots of Gopher were not examined carefully for seed color. The black-seeded form obtained was observed when notes on smut infection were taken. This black-seeded plant, like the other 14, segregated for seed color.

SUMMARY

Fourteen F_4 plants of the cross Gopher × Black Mesdag, belonging to families supposedly homozygous for white seed, were black seeded. Progeny tests of these black-seeded plants indicated that they were natural hybrids. In all, 5,132 F_4 plants from sources offering similar opportunities for natural crossing were examined. It it is assumed that as many reciprocal natural crosses occurred as there are crosses reported here, the percentage of natural hybrids between the black and white seeded forms was 0.54 per cent.

[3] GARBER, R. J., and QUISENBERRY, K. S. Op. cit.

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